

*Editor*

ROY WALDO MINER

**HYDROCORTISONE, ITS NEWER ANALOGS AND  
ALDOSTERONE AS THERAPEUTIC AGENTS**

BY

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JOSEPH W. JAILER



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# ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

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May 27, 1955

*Editor*

ROY WALDO MINER

## HYDROCORTISONE, ITS NEWER ANALOGS AND ALDOSTERONE AS THERAPEUTIC AGENTS\*

*Conference Chairman and Consulting Editor*

JOSEPH W. JAILER

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## Part I. Pharmacology of Hydrocortisone

### THE BIOSYNTHESIS OF ADRENAL STEROIDS\*

By Gregory Pincus

*The Worcester Foundation for Experimental Biology, Shrewsbury, Mass.*

#### *Introduction*

Intensive studies of the chemical transformations that adrenocortical tissue can effect with a large variety of steroid substrates have led to the isolation of a large number of products. Some of these products appear to be physiologically rational (*e.g.*, cortisol derived from progesterone), whereas others (*e.g.*, allopregnanedione derived from progesterone) can be assigned no physiological role. Because of these varied biosynthetic potencies of adrenocortical tissues, it is well, first, to determine what, in fact, is secreted by the adrenal cortex, and then to consider how the secretory product is manufactured. We have recently reviewed these two major problems in detail,<sup>1, 2</sup> so, in this paper, I should like to confine my attention to: (1) our latest evidence concerning the nature of the human adrenal secretory product; and (2) our present concept of the major operative biosynthetic pathways.

#### *The Secretory Product of the Human Adrenal Cortex*

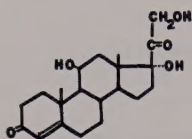
On initiating our studies of the secretory potency of the human adrenal several years ago, we planned very simply to repeat our original studies with the cow adrenals.<sup>3</sup> Fresh human glands taken at operation for adrenalectomy were perfused with citrated whole blood with and without added ACTH. Analysis of the perfusate for contained steroid was undertaken. We have elsewhere published in some detail the rather baffling results.<sup>4, 5</sup> First, the total steroid output of the isolated perfused glands was rather low and, second, there emerged, in contrast to our findings with the cow glands, no major easily identifiable product or products. Especially notable was our inability to detect significant amounts of corticosterone and/or cortisol that were consistently the outstanding products of cow gland perfusion and have been seen as major components of the steroid content of adrenal vein blood in a number of animal species.<sup>1, 2, 6</sup>

We were fortunate in being able to secure human adrenal vein blood at the operating table from two ACTH-treated patients being subjected to adrenalectomy, and we rather readily isolated and identified the compounds depicted in FIGURE 1.<sup>7</sup> Cortisol was by weight the preponderant crystalline product in the one case, and corticosterone in the other. Crystalline 11- $\beta$ -hydroxy-androstenedione was also obtained in both cases. In addition to these isolatable substances, we noted the presence of a number of other substances in our paper chromatograms.

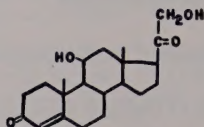
From these same two patients, we had obtained samples of peripheral venous

\* Original investigations described in this paper have been aided by grants from the United States Atomic Energy Commission, the American Cancer Society, Washington, D.C., and G. D. Searle and Company, Chicago, Ill.

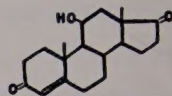
## COMPOUNDS IDENTIFIED IN HUMAN ADRENAL VEIN BLOOD



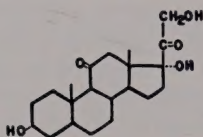
Compound "F"



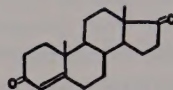
Compound "B"

11 $\beta$ -OH- $\Delta^4$ -Androstene-3,17-dione

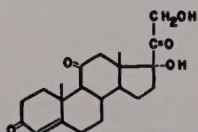
## COMPOUNDS TENTATIVELY IDENTIFIED



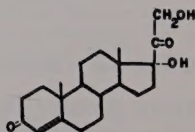
Tetrahydro "E"

 $\Delta^4$ -Androstene-3,17-dione

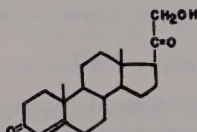
## COMPOUNDS SOUGHT BUT NOT FOUND



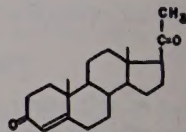
Compound "E"



Compound "S"



Desoxycorticosterone



Progesterone

FIGURE 1. Compounds isolated, indicated, and sought for in human adrenal vein blood.

blood as well as the adrenal vein blood. We therefore undertook a comparison of the steroid pattern in these two types of blood. Since most of the substances measured could not be identified by classical chemical methods, they were characterized on the basis of typical steroid color reactions and ultraviolet absorption. They fell into five classes, as indicated in TABLE 1. The data of this table demonstrate that, for both patients, the largest difference between peripheral and adrenal vein blood is in the content of ketolic  $\Delta^4$ -3 ketones, *i.e.* the substances characteristically adrenocortical. The data for the patient with breast cancer exhibit also increments over the peripheral blood in the adrenal vein content of other types of substances, notably in the nonketolic  $\Delta^4$ -3 ketones. The total steroid content of the adrenal vein blood of the prostatic cancer patient was 17.1 mg. per liter, that of the breast cancer patient, 50.7 mg. per liter, in contrast to 9.6 and 6.5 mg. per liter respectively in the peripheral blood.

These various compound types were analyzed into their individual constituents, with the result that a total of 50 different substances was seen one or more times.<sup>8, 9</sup> The substances as measured in the various classes are listed in TABLES 2, 3, and 4, on the basis of their running rates on paper in a propylene glycol:toluene system. The running rate of cortisol (compound No. 22, TABLE 3) is taken as 1.0, and all others are relative to this standard. It will be



TABLE 1

CONTENT (MG. PER LITER) OF VARIOUS STEROIDS IN THE PERIPHERAL AND ADRENAL VEIN BLOOD OF ACTH-TREATED PATIENTS

Compound type	♂—prostatic cancer		♀—breast cancer	
	Peripheral	Adrenal vein	Peripheral	Adrenal vein
$\alpha$ -ketols, saturated.....	3.02	2.17	2.81	8.29
Ketolic $\Delta^4$ -3-ketones.....	0.77	12.5	1.45	29.2
Ketolic ketones.....	1.06	1.35	1.41	0.33
Nonketolic $\Delta^4$ -3-ketones.....	1.64	1.45	0.22	9.24
Nonketolic ketones.....	3.11	0.64	0.60	3.61

TABLE 2

THE CONTENT (MG. PER LITER) OF THE SATURATED  $\alpha$ -KETOLS SEEN IN THE PERIPHERAL AND ADRENAL VEIN BLOOD OF TWO ACTH-INJECTED CANCEROUS PATIENTS

Compound No.	Running rate	Male—prostatic cancer		Female—breast cancer	
		Peripheral	Adrenal vein	Peripheral	Adrenal vein
1	0.05	0.17	—	—	0.03
2	0.10	0.43	—	0.07	—
3	0.14	0.91	0.02	0.59	—
4	0.23	0.07	1.80	—	—
5	0.29	0.57	0.52	—	0.02
6	0.34	0.18	0.23	—	0.95
7	0.70	0.16	—	0.31	—
8	1.05	—	0.40	0.12	0.05
9	1.83	0.13	0.08	0.01	3.26
10	2.39	—	0.16	0.36	—
11	3.35	0.06	—	—	2.28
12	4.54	0.06	0.02	—	—
13	6.19	—	0.16	0.09	0.89
14	8.35	0.29	0.38	—	—
15	11.3	—	0.09	0.10	0.76
16	34.2	—	0.16	—	—
17	44.6	—	1.40	—	—

noted that certain substances (*A*) are identifiable only in peripheral blood (*PV*) and not in adrenal vein (*AV*) blood (e.g. Nos. 22, 7, 19, 21, 24, 28, 36, 42, 43, 47), others (*B*) are seen in both types of blood sample but either higher concentration in *PV* or in about the same concentration in *PV* and *AV* (e.g. Nos. 2, 3, 5, 8, 12, 14, 31, 41, 46, 50). *A* and *B* may be taken as substances *not* secreted by the adrenal, since one should expect to find true secretory products present in *AV* either in much larger concentration than in *PV* or present in significant amount in *AV* and undiscernible in *PV*. On this basis, we have determined the probable secretory substances in the *AV* samples of these two patients and emerged with the data of TABLE 5. Some 13 to 17 substances are indicated as probable secretion products from the ACTH-stimulated adrenal. In our experiments with the isolated perfused cow adrenals, we deduced, on the basis of the increased production following ACTH administration, that some

TABLE 3

THE CONTENT (MG. PER LITER) OF VARIOUS STEROIDS IN PERIPHERAL AND ADRENAL VEIN BLOOD

Compound No.	Running rate	Male—prostatic cancer		Female—breast cancer	
		Peripheral	Adrenal vein	Peripheral	Adrenal vein
<i>α-ketolic, Δ<sup>4</sup>-3-ketones</i>					
18	0.013	—	0.60	1.05	—
19	0.355	0.02	—	—	—
20	0.62	0.04	0.06	—	6.35
21	0.75	0.18	—	—	—
22	1.0	0.10	8.50	0.40	6.38
23	1.48	0.12	0.94	—	—
24	5.81	0.004	—	—	—
25	8.25	0.29	2.4	—	13.33
26	10.7	—	—	—	2.54
27	14.5	—	—	—	0.59
<i>α-ketolic, saturated ketones</i>					
28	0.12	0.83	—	—	—
29	0.20	—	0.12	—	0.33
30	1.02	—	0.22	—	—
31	2.08	0.23	0.23	—	—
32	4.29	—	0.18	1.41	—
33	33.4	—	0.16	—	—

TABLE 4

THE CONTENT (MG. PER LITER) OF VARIOUS STEROIDS IN PERIPHERAL AND ADRENAL VEIN BLOOD

Compound No.	Running rate	Male—prostatic cancer		Female—breast cancer	
		Peripheral	Adrenal vein	Peripheral	Adrenal vein
<i>Nonketolic, <math>\Delta^4</math>-3-ketones</i>					
34	0.04	—	—	—	0.48
35	0.14	—	—	0.09	1.15
36	0.19	—	—	0.01	—
37	1.53	—	0.84	—	5.34
38	2.58	0.57	—	0.12	1.87
39	5.64	—	—	—	0.01
40	8.9	—	0.02	—	—
41	14.1	0.20	0.32	0.01	0.39
42	54.8	0.87	—	—	—
<i>Nonketolic, saturated ketones</i>					
43	0.06	—	—	0.02	—
44	0.20	0.14	—	—	—
45	0.40	—	—	—	0.05
46	1.68	1.01	0.40	0.16	—
47	8.30	0.48	—	—	—
48	17.7	0.04	0.04	—	3.05
49	25.5	—	0.04	—	—
50	55.0	1.47	0.19	0.96	0.51



TABLE 5  
THE NUMBER OF SUBSTANCES PROBABLY PRODUCED BY THE ADRENAL IN THE TWO CASES STUDIED

Type of compound	Male—prostatic cancer	Female—breast cancer
$\alpha$ -ketols, saturated.....	6	5
$\alpha$ -ketolic $\Delta^4$ -3-ketones.....	4	5
$\alpha$ -ketolic ketones.....	2-4	1
Nonketolic $\Delta^4$ -3-ketones.....	1	5
Nonketolic ketones.....	0	1
Totals.....	13-15	17

TABLE 6  
 $\alpha$ -KETOLS PRESENT IN BOVINE ADRENAL PERFUSATES BEFORE (NO ACTH) AND AFTER (ACTH) THE ADMINISTRATION OF ADRENOCORTICOTROPHIC HORMONE IN VITRO

Ketols	No ACTH	ACTH	$\Delta$ ACTH
Unk. 1-5.....	70	700	630
F.....	125	1100	975
Cort.....	200	1100	900
Unk. 6.....	35	{ 200	{ 150
E.....	20		
Unk. 7-9.....	40	250	210
A.....	{ 50	250	{ 500
Unk. 10.....		300	
DOC.....	50	150	100

15  $\alpha$ -ketols may be secreted (TABLE 6). These data suggest the secretion of 12 to 14  $\alpha$ -ketols in one subject and 11 in the other.

Among the indicated secretory products, we have identified, with some certainty, cortisol (No. 22), corticosterone (No. 25), 11- $\beta$ -OH-androstenedione (No. 37), androstenedione (No. 41), and tetrahydrocortisone (No. 4). There remain a number of others still to be identified, and their physiological significance is yet to be assessed. It is probable that the saturated compounds (*e.g.* tetrahydrocortisol) represent the action of nonspecific intra-adrenal hydrogenases on  $\Delta^4$ -3-ketones, and that their presence in larger amount in *AV* is an accidental consequence of the high concentration of their precursors. Nevertheless, there are a sufficient number of "typical" adrenal enzymatic transformations possible to lend credence to the possibility that the secretory product may contain, in fact, a notable aggregation of diverse substances having specific chemical constitutions. Let us, therefore, examine data on the biosynthetic potency of the adrenal cortex.

#### *Pathways of Adrenocorticosteroid Synthesis*

In FIGURE 2 we summarize the established intra-adrenal synthetic processes leading to the production of characteristic corticosteroids from cholesterol, the principal precursor. Details of the enzymatic mechanisms involved are discussed by Dorfman.<sup>10, 11</sup> I should like here to indicate some established

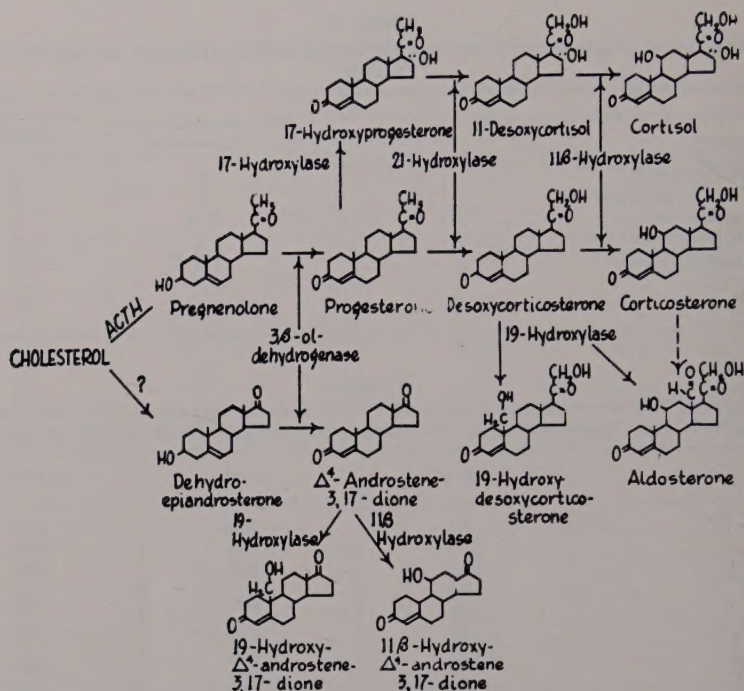


FIGURE 2. Steroidogenic processes in the adrenal cortex.

and some not so well established but logical consequences of the operation of these biosynthetic mechanisms.

First, it is now fairly well demonstrated that, on a quantitative basis, the major synthetic pathways from cholesterol are those eventuating in the production of cortisol and/or corticosterone. The evidence has come from quite diverse sources, *i.e.* from studies of adrenal vein blood, of isolated perfused adrenal glands, of adrenal slice and homogenate preparations.<sup>1, 2</sup> Furthermore, the administration of the key intermediates ( $\Delta^5$ -pregnenolone, progesterone) to isolated perfused glands or adrenal slices lead to cortisol and/or corticosterone as the dominant products.<sup>3, 12, 13</sup> An interesting unsolved mystery is why the gland secretes these characteristic corticosteroids in major amount rather than the five intermediates. Although the capacity of the gland to produce  $\Delta^5$ -pregnenolone and progesterone from cholesterol has been unequivocally established with the use of isotopic cholesterol,<sup>14, 10</sup> neither of these substances have yet been found in adrenal vein blood. A thorough study, not merely of turnover rates but also of possible intraglandular release mechanisms, is clearly indicated.

Second, the established processes indicated in FIGURE 2 suggest at least four other end-products of corticosteroidogenesis: (a) aldosterone; (b) 11- $\beta$ -hydroxy-androstenedione; (c) 19-hydroxyandrostenedione; and (d) 19-hydroxy-11-desoxy-corticosterone. Available evidence indicates that aldosterone is quanti-



tatively a minor product,<sup>15</sup> but its great physiologic potency establishes it as a most significant one. Our data on human adrenal vein blood indicate that, under ACTH stimulation, 11- $\beta$ -hydroxyandrostenedione accounts for 5 per cent to 10 per cent of the total steroid output (corticosterone plus cortisol account for 40 per cent to 60 per cent).

The recent discovery by Meyer of a 19-hydroxylating activity which acts upon dehydroepiandrosterone and  $\Delta^4$ -androstenedione<sup>16</sup> has been extended to the 21-carbon steroid by Hayano and Dorfman<sup>17</sup> and Levy and Kushinsky.<sup>18</sup> There is thus indicated a new mode of biosynthesis, the biological significance of which remains to be assessed. The fact that, hitherto, no 19-hydroxysteroids have been identified in either adrenal extracts or adrenal vein blood does not speak either for or against a functional significance of the 19-hydroxylating activity. The yields of 19-hydroxysteroid thus far obtained from perfusion and tissue incubation studies vary from a fraction of 1 per cent to about 3 per cent, so that it is likely that such compounds may be found as adrenal secretory products.

Third, when one considers the various possible permutations consequent on established enzymatic processes, it is entirely likely that other biosynthetic products will be discovered. Unless very narrow substrate enzyme relationships exist, we should expect to find such compounds as 17-hydroxyaldosterone and its 19-carbon derivative, 19-hydroxycortisol, 19-hydroxycorticosterone, and so on. The array of possible adrenocortical products is staggering, and it may be that we have been underestimating the steroidogenic potency of the adrenal cortex.

There is time to touch but briefly on some of the other significant aspects of adrenal steroid biosynthesis. Our concept of the genesis from cholesterol of the major end products cortisol and/or corticosterone involves an ACTH-labile scission of the side chain of cholesterol at carbon-20, eventuating in the production of  $\Delta^5$ -pregnenolone and then a series of ACTH-independent enzymatic reactions. We are still uncertain of the factors controlling the side chain scission at carbon-17, which yields the 19-carbon steroids. Even the action of ACTH requires much elucidation before the true mechanism can be understood. Finally, when we consider the substantial evidence for a "total synthesis" in the adrenal of adrenocorticoids by a pathway independent of cholesterol (and of ACTH), it is clear that we have much more to discover than is now known. The unraveling of the complex of processes involved is, however, inevitable, and we can look forward to an account of corticosteroidogenesis that will form a remarkable chapter in the history of biochemistry.

### References

1. PINCUS, G. 1954. The biosynthesis of adrenal steroids. *Progress in Allergy*. **4**: 198.
2. HECHTER, O. & G. PINCUS. 1954. Genesis of the adrenocortical secretion. *Physiol. Revs.* **34**: 459.
3. HECHTER, O., L. ZAFFARONI, R. P. JACOBSEN, H. LEVY, R. W. JEANLOZ, V. SCHENKER & G. PINCUS. 1951. The nature and biogenesis of the adrenal secretory product. *Recent Progress in Hormone Research*. **6**: 215.
4. PINCUS, G., E. B. ROMANOFF & L. P. ROMANOFF. 1953. Current status of corticosteroid metabolism in man. *Ciba Foundation Colloquia on Endocrinol.* **7**: 240.

5. PINCUS, G. 1954. Transformations of steroids by tissues. Proc. 2nd Natl. Cancer Conf. : 1494.
6. BUSH, I. E. 1953. Species differences and other factors influencing adrenocortical secretion. Ciba Foundation Colloquia on Endocrinol. **7**: 210.
7. ROMANOFF, E. B., P. HUDSON & G. PINCUS. 1953. Isolation of hydrocortisone and corticosterone from human adrenal vein blood. J. Clin. Endocrinol. and Metab. **12**: 1546.
8. PINCUS, G. & E. B. ROMANOFF. 1955. The synthesis of corticosteroids by the human adrenal cortex. Ciba Foundation Colloquia on Endocrinol. In press.
9. PINCUS, G. 1954. Aspects du métabolisme des Stéroïdes Hormonaux. Actualités Biochimiques. Masson and Cie. Paris, France.
10. DORFMAN, R. I., M. HAYANO, R. HAYNES & K. SAVARD. 1953. The *in vitro* synthesis of adrenal cortical steroids. Ciba Foundation Colloquia on Endocrinol. **7**: 191.
11. DORFMAN, R. I. 1954. The metabolism of adrenal steroids. In Adrenal Cortex. Ralli, Ed. Josiah Macy Jr. Foundation, New York.
12. LEVY, H., R. W. JEANLOZ, R. P. JACOBSEN, O. HECHTER, V. SCHENKER & G. PINCUS. 1950. The chemical transformations of progesterone by adrenal perfusion. Abstr. 118th Meet. Am. Chem. Soc. : 29C.
13. LEVY, H., R. W. JEANLOZ, R. P. JACOBSEN, O. HECHTER, V. SCHENKER & G. PINCUS. 1954. Chemical transformation of steroids by adrenal perfusion. Progesterone, 17 $\alpha$ -hydroxyprogesterone, and pregn-5-en-3 $\beta$ -ol-20 one. J. Biol. Chem. **211**: 867.
14. SABA, N., O. HECHTER & D. STONE. 1954. The conversion of cholesterol to pregnenolone in bovine adrenal homogenates. J. Am. Chem. Soc. **76**: 3862.
15. SIMPSON, S. A. & J. F. TAIT. 1955. Recent progress in methods of isolation, chemistry and physiology of aldosterone. Recent Progress in Hormone Research. **11**.
16. MEYER, A. 1955. 19-hydroxylation of  $\Delta^4$ -androstene-3, 17-dione and dehydroepiandrosterone by bovine adrenals. Experientia. In press.
17. HAYANO, M. & R. I. DORFMAN. 1955. The conversion of desoxycorticosterone to 19-hydroxy-11-desoxycorticosterone by adrenal homogenate residues. Arch. Biochem. Biophys. In press.
18. LEVY, H. & S. KUSHINSKY. 1955. The isolation of 19-hydroxy-11-desoxycorticosterone and an unknown active mineralocorticoid from bovine adrenal perfusions of progesterone. Arch. Biochem. Biophys. In press.



# SPECIAL ASPECTS OF ADRENOCORTICAL STEROID METABOLISM

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Since the anabolism and catabolism of adrenocortical steroids has been reviewed recently,<sup>1-6</sup> this paper will be concerned only with special aspects of steroid metabolism. The following topics will be discussed: (1) ring A reduction of  $\Delta^4$ -3-ketones; (2) conversion of  $17\alpha$ -hydroxy- $C_{21}$  steroids to 17-desoxy- $C_{21}$  steroids; (3) conversion of  $C_{21}$  to  $C_{19}$  steroids (*in vitro*); and (4) new cortisol metabolites.

## *Ring A Reduction of $\Delta^4$ -3-Ketones*

The biologically active adrenocortical steroids contain  $\Delta^4$ -3-ketone groupings in ring A which appear to be intimately associated with their actions. In metabolism, this grouping is reduced, in large measure, at carbons 3, 4, and 5 and, when this occurs, four stereoisomeric forms are possible, due to the various forms that can be produced at carbons 3 and 5. In previous publications,<sup>3, 4, 5, 7</sup> it was suggested that the type of stereoisomer that appears as a result of the *in vivo* reduction of the 4,5 grouping, in humans, is dependent upon the nuclear substitution on the steroid nucleus. Briefly,  $C_{19}O_2$  steroids, during *in vivo* metabolism in humans, are reduced to the  $5\alpha$  (androsterone) and  $5\beta$  (etiocholanone) stereoisomeric forms in essentially equal proportions.  $C_{19}$  steroids possessing oxygen functions at carbon 11 are reduced primarily to the  $5\alpha$  forms while the presence of the side chain ( $C_2$ ), with or without oxygen substitution at carbon 11, orients the reduction of the  $\Delta^4$  group predominantly to the  $5\beta$  (pregnane) form. In earlier reports, it was pointed out that these generalizations were based on a limited number of studies, and that further data were needed. It was further pointed out that these ratios ( $5\beta/5\alpha$ ) may be modified by the age, sex, or physiological status of the individual.

Recent reports have strengthened the validity of the generalizations concerning the ring A reduction of  $C_{21}$  steroids. New experiments,<sup>8, 18</sup> however, involving conversion of testosterone to androsterone ( $5\alpha$ ) and etiocholanolone ( $5\beta$ ) in humans have demonstrated relatively wide variations in ratio of  $5\beta$  to  $5\alpha$  products at different time intervals after administration. The mean overall ratio for nine experiments was 0.8 ( $5\beta/5\alpha$ ), in contrast to the values of 1.29 previously reported.

Studies on the reduction of  $C_{21}$   $\Delta^4$ -3-ketones to the dihydro and tetrahydro forms have been extended by various investigators. Richardson and his collaborators<sup>9</sup> have reported that all of the six known adrenocortical hormones are reduced primarily to the  $5\beta$  stereoisomer. Actually, these workers have isolated the  $3\alpha,5\beta$  tetrahydro forms of desoxycorticosterone, 11-desoxycortisol, corticosterone, 11-dehydrocorticosterone, in addition to those of cortisone and cortisol.

Engel *et al.*<sup>10</sup> administered large doses of corticosterone to one patient and found a concentration of  $5\alpha$  reduced products equal to those of the  $5\beta$  stereoi-

somers. This finding is unique since, in all other studies involving the reduction of the  $\Delta^4$  group in  $C_{21}$  steroids, the principal urinary products have always been found to be in the  $5\beta$  series. No reason for this finding is evident at the moment.

Until recently, most reductive changes at carbons 4 and 5 of  $C_{21}$  steroids by liver and adrenal tissue under *in vitro* conditions yielded only the  $5\alpha$  stereoisomer. Studies of this kind have been reported by Schneider, employing desoxycorticosterone;<sup>11, 12</sup> by Caspi *et al.*,<sup>13, 14, 15</sup> employing cortisone and cortisol; and by Forchielli *et al.*,<sup>16</sup> using 11-desoxycortisol. These results are in contrast to those found by the *in vivo* techniques where, primarily,  $5\beta$  stereoisomers in urine were found after administration of  $C_{21}$  steroids to humans.

The apparent inconsistency of finding  $5\alpha$  reduction by *in vitro* methods, as compared to the  $5\beta$  reduction *in vivo*, can be understood better in the light of more recent reports. Significant newer findings include the work of Horwitt and Segaloff,<sup>17</sup> who demonstrated that progesterone, incubated with rabbit liver, yielded the  $5\beta$  stereoisomer, pregnane- $3\alpha, 20\alpha$ -diol, and of Tompkins and Isselbacher,<sup>18</sup> who showed that cortisone may be converted to the  $3\alpha, 5\beta$  tetrahydrocortisone derivative by a supernatant derived from a rat-liver homogenate.

Two recent studies using liver homogenates have indicated that both the  $5\alpha$  and  $5\beta$  reducing enzyme systems are indeed present in liver. Taylor<sup>19</sup> employed an homogenate derived from rabbit liver that was capable of reducing progesterone to both  $5\alpha$  and  $5\beta$  derivatives. The  $5\beta$  steroid,  $3\alpha$ -hydroxypregnan-20-one, and three  $5\alpha$  steroids, allopregnan-3,20-dione,  $3\beta$ -hydroxyallopregnan-20-one, and  $3\alpha$ -hydroxyallopregnan-20-one were isolated. This study marks the first demonstration that both enzymes ( $5\alpha$  and  $5\beta$ ) are present in the same tissue preparation.

Forchielli *et al.*,<sup>16</sup> who previously had demonstrated that 11-desoxycortisol is converted to  $5\alpha$  (allopregnane) derivatives purified their preparation further and now have observed both  $5\alpha$  and  $5\beta$  reduction. The original preparation consisted of a supernatant derived by centrifuging a rat-liver homogenate at 6000 g. The supernatant employed in the first studies has been subjected to 78,000 g., and the new supernatant contains the  $5\beta$  reducing enzyme system. The particulate matter, which represents the material separable between 6000 g. and 78,000 g., contains the  $5\alpha$  reducing enzyme system.<sup>20</sup> In this latest study, 11-desoxycortisol incubated with the 78,000 g. supernatant yielded  $3\beta, 17\alpha, 21$ -hydroxypregnan-20-one and  $3\alpha, 17\alpha, 21$ -trihydroxypregnan-20-one, while incubation of the same substrate with the 78,000 g. residue has yielded  $3\alpha, 17\alpha, 21$ -trihydroxypregnan-20-one.

#### *Conversion of $17\alpha$ -Hydroxy- $C_{21}$ Steroids to $17$ -Desoxy- $C_{21}$ Steroids*

Ungar *et al.*<sup>21</sup> administered  $17\alpha, 21$ -dihydroxypregnan-3,20-dione to a postmenopausal woman and found an increased excretion of pregnane- $3\alpha, 20\alpha$ -diol. The yield of the diol was small, and the possibility of stimulating the production of pregnane- $3\alpha, 20\alpha$ -diol precursors was suggested. In a second study, Rosset *et al.*<sup>22</sup> have found that the administration of 21-desoxycortisol and 17-hydroxyprogesterone to Addisonian patients resulted in the excretion of in-

creased quantities of 17-desoxy-C<sub>21</sub> steroids. 21-Desoxycortisone yielded 3 $\alpha$ -hydroxypregnane-11,20-dione, while the *in vivo* metabolism of 17 $\alpha$ -hydroxyprogesterone led to the increased excretion of 3 $\alpha$ -hydroxypregnan-20-one.

According to a third study, reported by Miller and Axelrod,<sup>23</sup> the perfusion of cortisone through a cirrhotic rat liver yielded 6 $\beta$ -hydroxydesoxycorticosterone, which again illustrates the reaction involving the formation of a 17-desoxy form from the 17-hydroxy steroid.

The finding of 17-desoxy-20-ketone compounds in urinary extracts may be due to artifact formation from 17,20-dihydroxy steroids by the action of mineral acid and heat. The isolation of the 17-desoxy-20-hydroxy derivative, on the other hand, seems more convincing.

If this conversion (17-hydroxy to 17-desoxy) actually occurs in the body, a serious complication is added to the quantitative study of urinary metabolites as indicators of adrenocortical hormones. As illustrated in FIGURE 1, about four different types of compounds would be expected to yield 20-oxygenated metabolites with no oxygen substitution at 17 and 21. This could happen because, in addition to the formation of 17-desoxy steroids from 17 $\alpha$ -hydroxy steroids, it is known that 21-hydroxy compounds can be reduced to 21-desoxy compounds.<sup>21, 24-28</sup>

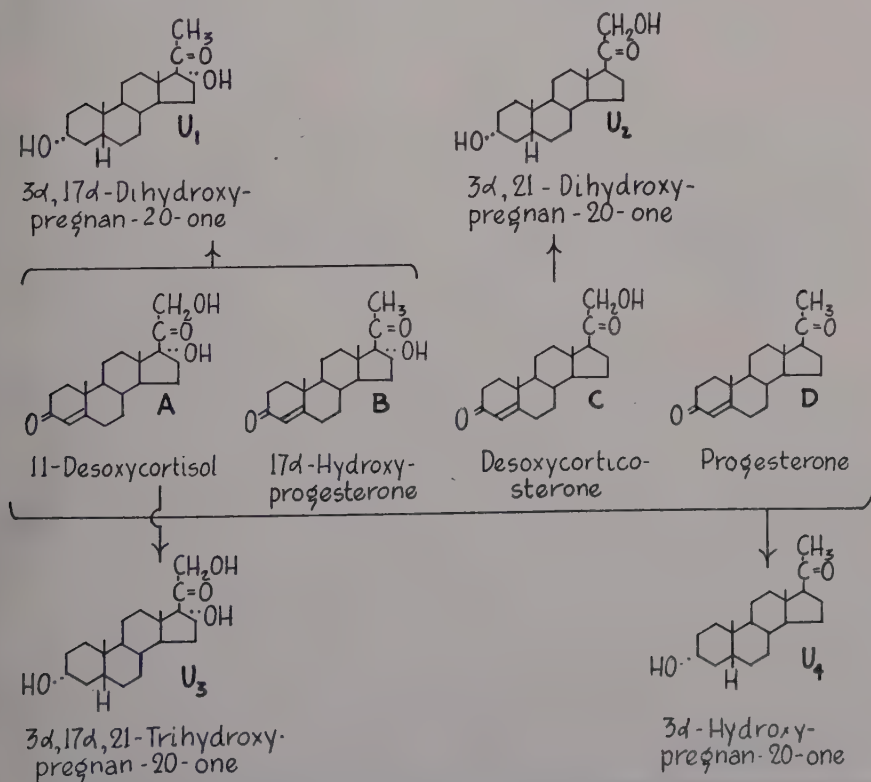


FIGURE 1. Possible metabolism of 11-desoxycortisol, 17 $\alpha$ -hydroxyprogesterone, desoxycorticosterone, and progesterone, *in vivo*.



If the conversion of 17-hydroxy steroids to 17-desoxy steroids actually occurs, the scheme outlined in FIGURE 1 may be helpful in relating urinary steroids to the tissue precursors. FIGURE 1 lists the four compounds in the 11-desoxy series that may be converted to  $3\alpha$ -hydroxypregnan-20-one ( $U_4$ ) and, in turn, to pregnane- $3\alpha,20\alpha$ -diol (not listed in FIGURE 1). Compounds A through D are present in adrenal tissues, and the urinary metabolites are designated as  $U_1$ ,  $U_2$ ,  $U_3$ , and  $U_4$ . To determine individual components, we may assume a constant relationship between the quantity of hormone produced in the tissue and the amount excreted in the urine. It is fully recognized that this may vary, and experiments designed to determine these variations must be done. If, however, this assumption is used, then:

$U_1$  would be a measure of production of components A + B;

$U_2$  would be a measure of production of component C;

$U_3$  would be a measure of production of component A;

$U_4$  would be a measure of production of components A + B + C + D.

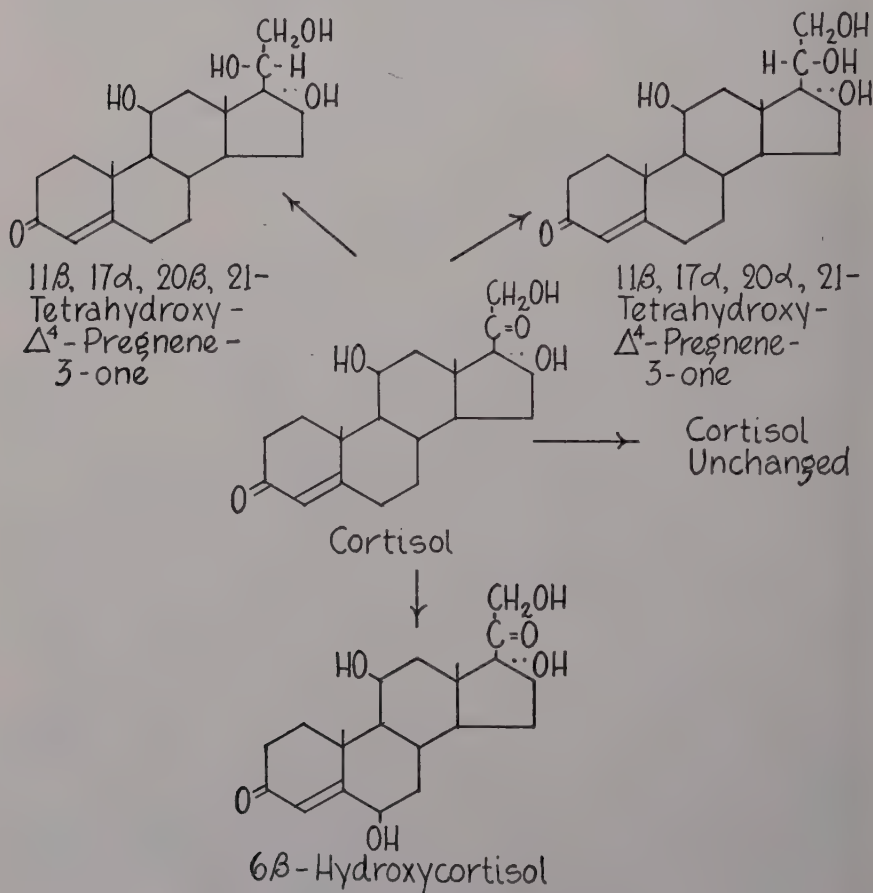


FIGURE 2. Metabolism of cortisol in guinea pigs, *in vivo*.

Therefore,  $U_1 - U_3$  would be a measure of production of B, and  $U_4 - U_1 - U_2$  would be a measure of production of D.

### *Conversion of $C_{21}$ to $C_{19}$ Steroids (in Vitro)*

The rupture of carbon to carbon bond between carbon atoms 17 and 20 forming  $C_{19}$  steroids is well established under various *in vivo* conditions,<sup>21, 27-30</sup> and by perfusion experiments.<sup>13-15</sup> Recently, a relatively purified rat liver enzyme system has been prepared that carried out this reaction *in vitro*. Forchielli *et al.*<sup>16</sup> have demonstrated that the supernatant prepared from a rat liver homogenate (6000 g.) contained an enzyme system capable of converting 11-desoxycortisol and/or its  $C_{21}$  metabolites to corresponding 17-ketosteroids. The two 17-ketosteroids,  $\Delta^4$ -androstene-3,17-dione and androsterone, were isolated and identified from the incubation mixture.

### *New Cortisol Metabolites*

Fukushima *et al.*<sup>37</sup> administered  $C^{14}$ -labeled cortisol to humans and identified pregnane- $3\alpha, 11\beta, 17\alpha, 20\alpha, 21$ -pentol (cortol),  $3\alpha, 17\alpha, 20\alpha, 21$ -tetrahydroxypregnan-11-one (cortolone), pregnane- $3\alpha, 11\beta, 17\alpha, 20\beta, 21$ -pentol ( $\beta$ -cortol), and  $3\alpha, 17\alpha, 20\beta, 21$ -tetrahydroxypregnan-11-one ( $\beta$ -cortolone). The same four steroids were identified in human urines after ACTH administration. These newly discovered cortisol metabolites are all of the  $5\beta$  series and are reported to account for 30 per cent of the administered steroid.

Recent studies on guinea pig urine revealed the presence of cortisol<sup>31, 32, 33</sup> and  $6\beta$ -hydroxycortisol.<sup>34</sup>  $6\beta$ -Hydroxycortisol, together with  $11\beta, 17\alpha, 20\beta, 21$ -tetrahydroxy- $\Delta^4$ -pregnen-3-one and  $11\beta, 17\alpha, 20\alpha, 21$ -tetrahydroxy- $\Delta^4$ -pregnen-3-one are metabolites of cortisol<sup>35</sup> (FIGURE 2).  $6\beta$ -Hydroxycortisol has now been isolated and identified in human urines. Fresh urine derived from a Cushing's syndrome patient who received cortisol intravenously yielded the  $6\beta$ -hydroxy steroid. The same steroid was identified in late human pregnancy urine.<sup>36</sup>

### *References*

1. PINCUS, G. 1954. The biosynthesis of adrenal steroids. *In* Progress in Allergy. **4**: 199. P. Kallos & S. Karger, Eds. New York, N.Y.
2. HECHTER, O. & G. PINCUS. 1954. Genesis of the adrenocortical secretion. *Physiol. Revs.* **34**: 459.
3. DORFMAN, R. I. 1954. Adrenocortical steroid metabolism. 5th Conf. on the Adrenal Cortex. Josiah Macy Jr. Found., New York, N. Y.
4. DORFMAN, R. I. 1954. Neutral steroid hormone metabolites. *Rec. Progr. Hormone Research.* **9**: 5.
5. DORFMAN, R. I. & F. UNGAR. 1953. Metabolism of Steroid Hormones. Burgess. Minneapolis, Minn.
6. LIEBERMAN, S. & S. TEICH. 1953. Recent trends in the biochemistry of the steroid hormones. *Pharmacol. Rev.* **5**: 285.
7. DORFMAN, R. I. 1954. *In vivo* metabolism of neutral steroid hormones. *J. Clin. Endocrinol. & Metab.* **14**: 318.
8. FUKUSHIMA, D. K., K. DOBRINER & T. F. GALLAGHER. 1954. Studies with testosterone-d in normal men. *J. Biol. Chem.* **206**: 845.
9. RICHARDSON, E. M., J. C. TOUCHSTONE & F. C. DOHAN. 1954. Paper chromatographic studies of the urinary alpha-ketolic metabolites of adrenal cortical hormones. *Federation Proc.* **13**: 118.
10. ENGEL, L. L., P. CARTER & M. J. SPRINGER. 1954. Isolation from urine of ketonic metabolites of administered corticosterone. *Federation Proc.* **13**: 204.

11. SCHNEIDER, J. J. & P. M. HORSTMANN. 1951. Effects of incubating desoxycorticosterone with various rat tissues. *J. Biol. Chem.* **191**: 327.
12. SCHNEIDER, J. J. 1952. Conversion of desoxycorticosterone to four allopregnane metabolites by rat liver *in vitro*. *J. Biol. Chem.* **199**: 235.
13. CASPI, E., H. LEVY & O. HECHTER. 1953. Cortisone metabolism in liver. II. Isolation of certain cortisone metabolites. *Arch. Biochem. Biophys.* **45**: 169.
14. CASPI, E. & O. HECHTER. 1954. Corticosteroid metabolism in liver. III. Isolation of additional cortisone metabolites. *Arch. Biochem. Biophys.* **52**: 478.
15. CASPI, E. 1955. Transformation products resulting from cortisone and cortisol perfusion through rat livers. Ph.D. Thesis. Dept. of Chem., Clark Univ., Worcester, Mass.
16. FORCHIELLI, E., H. ROSENKRANTZ & R. I. DORFMAN. 1955. Metabolism of 11-desoxycortisol *in vitro*. *J. Biol. Chem.* In press.
17. HORWITT, B. N. & A. SEGALOFF. 1954. *In vitro* conversion of progesterone to pregnane-3 $\alpha$ ,20 $\alpha$ -diol. *Federation Proc.* **13**: 232.
18. TOMPKINS, G. & K. J. ISSELBACHER. 1954. Enzymatic reduction of cortisone. *J. Am. Chem. Soc.* **76**: 3100.
19. TAYLOR, W. 1954. Progesterone metabolism by rabbit liver *in vitro*. *Biochim. et Biophys. Acta.* **15**: 592.
20. FORCHIELLI, E. & R. I. DORFMAN. 1955. Unpublished results.
21. UNGAR, F., J. W. DAVIS, H. ROSENKRANTZ & R. I. DORFMAN. 1954. Metabolism of pregnane-17 $\alpha$ ,21-diol-3,20-dione and pregnane-17 $\alpha$ ,21-diol-3,11,20-trione *in vivo*. *J. Biol. Chem.* **207**: 375.
22. ROSSELET, J. P., M. FURMAN, S. LIEBERMAN & J. W. JAILER. 1954. *In vivo* conversion of C<sub>21</sub>-17 $\alpha$ -hydroxylated steroid to C<sub>21</sub>-17-desoxymetabolites. *Science.* **120**: 788.
23. MILLER, L. L. & L. R. AXELROD. 1954. Cortisone metabolism in the perfused normal and experimental cirrhotic rat liver. *Metabolism, Clin. and Exptl.* **3**: 438.
24. HOFFMAN, M. M., V. E. KAZMIN & J. S. L. BROWNE. 1943. The excretion of pregnanediol following the administration of desoxycorticosterone acetate to rabbits. *J. Biol. Chem.* **147**: 259.
25. HORWITT, B. N., R. I. DORFMAN, R. A. SHIPLEY & W. R. FISH. 1944. Metabolism of the steroid hormones. IV. Conversion of desoxycorticosterone to pregnanediol-3( $\alpha$ ),20( $\alpha$ ) in man and in the chimpanzee. *J. Biol. Chem.* **155**: 213.
26. MASON, H. L. 1948. Metabolites of 11-dehydrocorticosterone: pregnane-3( $\alpha$ ),20-diol-11-one. *J. Biol. Chem.* **172**: 783.
27. DOBRINER, K. & S. LIEBERMAN. 1952. The metabolism of steroid hormones in humans. *Ciba Foundation Colloquia on Endocrinology.* **2**: 381. J. & A. Churchill. London, England.
28. BURSTEIN, S., K. SAVARD & R. I. DORFMAN. 1953. The *in vivo* metabolism of cortisone. *Endocrinology.* **52**: 448.
29. BURSTEIN, S., K. SAVARD & R. I. DORFMAN. 1953. The *in vivo* metabolism of hydrocortisone. *Endocrinology.* **53**: 88.
30. BURSTEIN, S., K. SAVARD & R. I. DORFMAN. 1953. The *in vivo* metabolism of 21-desoxycortisone. *Endocrinology.* **53**: 267.
31. NADEL, E. M. & J. J. SCHNEIDER. 1952. Excretion of formaldehydegenic (FG) substances by normal and scorbutic guinea pigs. *Endocrinology.* **51**: 5.
32. BURSTEIN, S. & R. I. DORFMAN. 1954. Hydrocortisone in normal guinea pig urine: isolation and quantitative determination. *J. Biol. Chem.* **206**: 607.
33. BURSTEIN, S., R. I. DORFMAN & E. M. NADEL. 1954. Isolation of excreted corticosteroids in the guinea pig in late scurvy. *Federation Proc.* **13**: 188.
34. BURSTEIN, S., R. I. DORFMAN & E. M. NADEL. 1955. Corticosteroids in the urine of normal and scorbutic guinea pigs: isolation and quantitative determination. *J. Biol. Chem.* In press.
35. BURSTEIN, S. & R. I. DORFMAN. 1955. Steroid metabolism in guinea pigs. I. *In vivo* metabolism of cortisol. *J. Biol. Chem.* In press.
36. BURSTEIN, S., R. I. DORFMAN & E. M. NADEL. 1954. 6 $\beta$ -Hydroxycortisol—a new steroid in human urine. *Arch. Biochem. Biophys.* **53**: 307.
37. FUKUSHIMA, D. K., N. S. LEEDS, H. L. BRADLOW, T. H. KRITCHEVSKY, M. B. STOKEM & T. F. GALLAGHER. 1955. The characterization of four new metabolites of adrenocortical hormones. *J. Biol. Chem.* **212**: 449.
38. FUKUSHIMA, D. K., H. L. BRADLOW, K. DOBRINER & T. F. GALLAGHER. 1954. The fate of testosterone infused intravenously in man. *J. Biol. Chem.* **206**: 863.



# THE PHYSIOLOGICAL DISPOSITION AND METABOLIC FATE OF HYDROCORTISONE IN MAN

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## *Introduction*

Hydrocortisone has been used extensively, clinically, since 1950. Knowledge of its physiological distribution, however, is incomplete. Until now, little information has been available on the following fundamental questions: how long after administration to man or animals in health or in a disease state do corticosteroids remain in the circulation; in what manner are they distributed in the body fluids; how rapidly are they metabolized; to what extent are they conjugated; and, in what manner and at what rate are they excreted in the urine and feces? The development of a simple and sensitive method for the estimation of hydrocortisone in biological materials has permitted studies of its absorption, excretion, distribution, and rate of metabolic transformation. The present report describes the metabolic fate and physiological disposition of hydrocortisone in man.

## *Materials and Methods*

The hydrocortisone used in these studies was administered orally or intravenously in either trace quantities (100 to 700 micrograms) as C-4, C<sup>14</sup>-labeled material\* (1.54 millicuries per millimole or, in pharmacological quantities (50 to 600 mgm.). The intravenous administrations were made to fasting subjects in sterile solutions containing 5 per cent dextrose and 5 per cent ethanol.

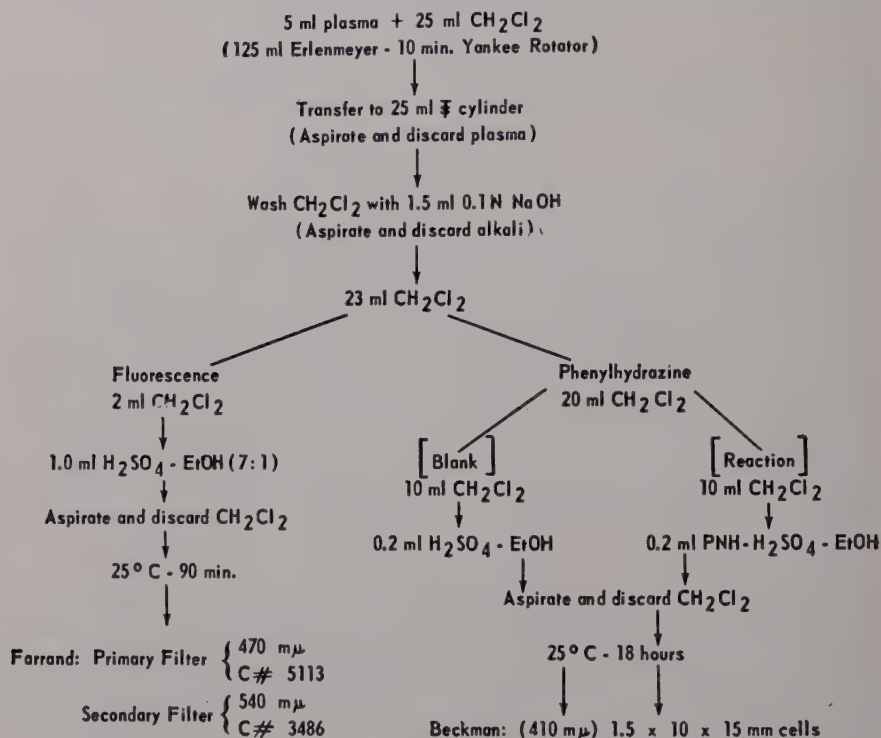
TABLE 1 shows the extraction scheme and methods of analysis used for the steroids in plasma following their infusion. Depending upon the structure of the steroid, it was possible to use a phenylhydrazine colorimetric analysis, a fluorometric analysis, or both. These are respectively modifications<sup>1</sup> of previously published methods of Silber and Porter<sup>2</sup> and of Sweat.<sup>3</sup> Colorimetric analysis of the urinary steroids was made by the phenylhydrazine method with or without the use of bacterial  $\beta$ -glucuronidase (Sigma) hydrolysis prior to dichloromethane extraction with 10 volumes of solvent.

## *Plasma Steroid Studies*

When 200 mgm. of hydrocortisone free alcohol was infused into normal subjects over a 20 to 30 minute period, and blood samples were collected at intervals of 20 to 30 minutes following the termination of the infusion and assayed for the free steroid content of the plasma, it was found that a plot on semilogarithmic paper of the steroid concentration versus time gave a straight line. The biologic half life of the hydrocortisone was found to range from 95 to 130 minutes in 18 normal subjects, with a mean of 115 minutes (FIGURE 1). The

\* Obtained from the Endocrinology Study Section of the National Institutes of Health, United States Public Health Service, Bethesda, Md.

TABLE 1  
DETERMINATION OF PLASMA STEROIDS



level of steroid, at zero time, was found to range from 200 to 300 micrograms per cent.

We have attempted to prove that the material in plasma after infusion is indeed hydrocortisone. The following evidence supports this conclusion: (1) countercurrent separation (8 and 16 tube transfers) of the dichloromethane extract of plasma obtained 90 minutes following termination of the infusion shows a distribution of the phenylhydrazine reacting material that is nearly identical with that of pure hydrocortisone and of hydrocortisone-4- $\text{C}^{14}$ ; (2) the curves of disappearance of phenylhydrazine reacting material and of fluorescent material show identical slopes; and (3) a small quantity of hydrocortisone-4- $\text{C}^{14}$  was added to the dichloromethane extract of a plasma sample obtained 90 minutes following termination of the infusion and the specific activity of the phenylhydrazine reacting material was determined. On comparing this specific activity to that obtained on the eluate of the hydrocortisone after paper chromatography of this same dichloromethane extract, very nearly identical specific activity data are obtained.

When hydrocortisone was infused into patients with liver disease (moderately severe cirrhosis), it was found in all eight cases (FIGURE 2) that the rate of its metabolism was very much slower than normal (biologic half times of 160 to

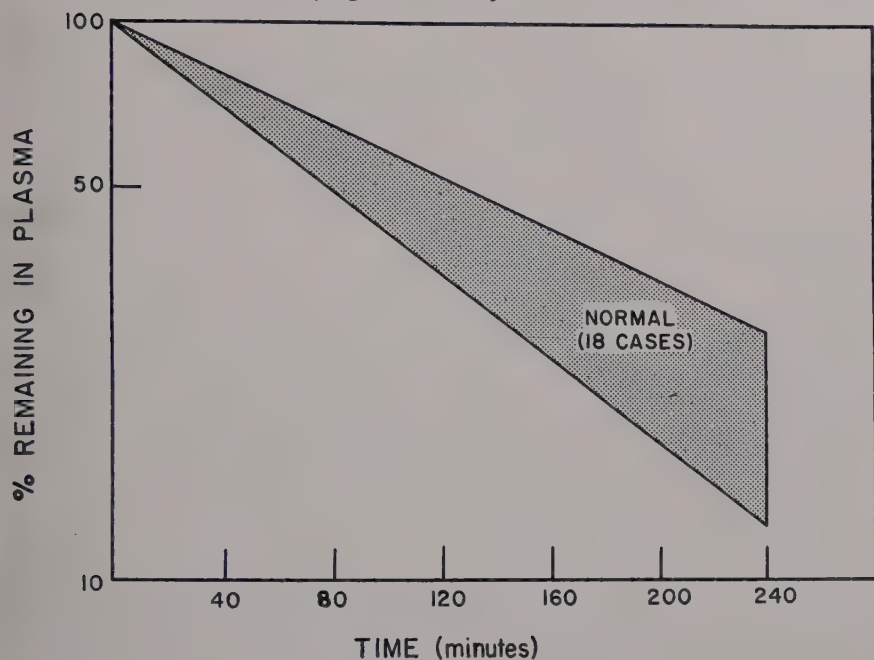


FIGURE 1. Disappearance of hydrocortisone from plasma in normal subjects.

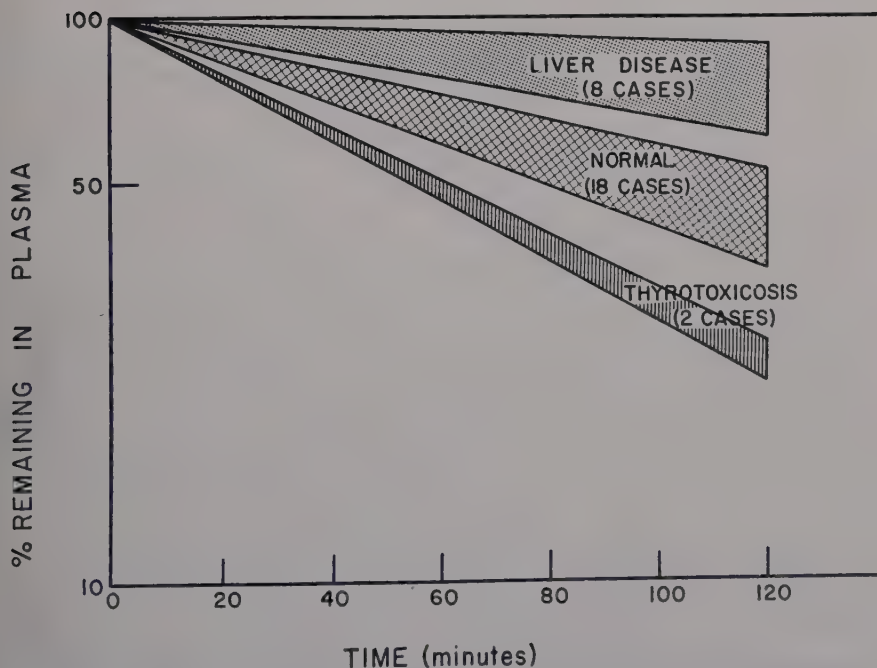


FIGURE 2. Disappearance of hydrocortisone from plasma in normal subjects and in patients with liver disease and thyrotoxicosis.



TABLE 2

HALF TIMES OF DISAPPEARANCE OF VARYING CONCENTRATIONS OF HYDROCORTISONE  
FOLLOWING INTRAVENOUS ADMINISTRATION

Hydrocortisone administered	Phenylhydrazine	Fluorometric	Specific activity of hydrocortisone
	<i>min.</i>	<i>min.</i>	<i>min.</i>
1 mg. C <sup>14</sup>	—	—	112
100 mg.	115	—	—
300 mg.	110	106	—
600 mg.	100	—	—

800 minutes). Similar studies in two cases of thyrotoxicosis have shown just the opposite—a more rapid rate of hydrocortisone metabolism. These findings in liver disease and in thyrotoxicosis are of special interest because, in the presence of a normal level of hydrocortisone in the plasma, it can be postulated that the turnover rate (or daily endogenous production) of hydrocortisone will be decreased in liver disease and increased in hyperthyroidism. Studies along this line are now in progress.

That the disappearance of hydrocortisone from the plasma is a first order reaction related only to its metabolism is suggested from the fact that a single straight line is obtained when plotted on semilogarithmic paper. TABLE 2 demonstrates the biologic half times obtained following the infusion of various concentrations of hydrocortisone.

Other steroids closely related to hydrocortisone, such as cortisone, epihydrocortisone, 20-hydroxy hydrocortisone,\* corticosterone,\* epicorticosterone,\* and tetrahydrocortisone have been studied in normal subjects and in patients with liver disease. In all cases, the biologic half times have been in the range of 50 to 70 minutes in both normals and patients with liver disease. Thus, the enzyme system in the liver responsible for the metabolism of hydrocortisone seems to be selectively impaired in liver disease.

Following the intravenous administration of tracer doses of hydrocortisone-4-C<sup>14</sup> and determination of the biologic half life of the dichloromethane-extracted plasma radioactivity, values in the range of 70 to 90 minutes were obtained. A fraction (approximately 50 per cent) of the total radioactivity in the dichloromethane-extracted plasma represents metabolites of hydrocortisone. This probably contributes to the shortened biologic half times.

Presumably, the dichloromethane extract contains no conjugated steroid. There are radioactive metabolites of hydrocortisone, however, that remain in the aqueous residue as glucuronide conjugates after dichloromethane extraction of plasma. FIGURE 3 shows how the percentage of the sum of the free and hydrolyzed fraction present as glucuronide increases with time and how, at 6 hours, this fraction represented 90 per cent of the total.

By determination of the counts per minute present in the dichloromethane extract of plasma at zero time by extrapolation of the curve of disappearance of radioactivity, a space of 20 to 25 liters was obtained. This is a volume only

\* Assayed by fluorescence method.

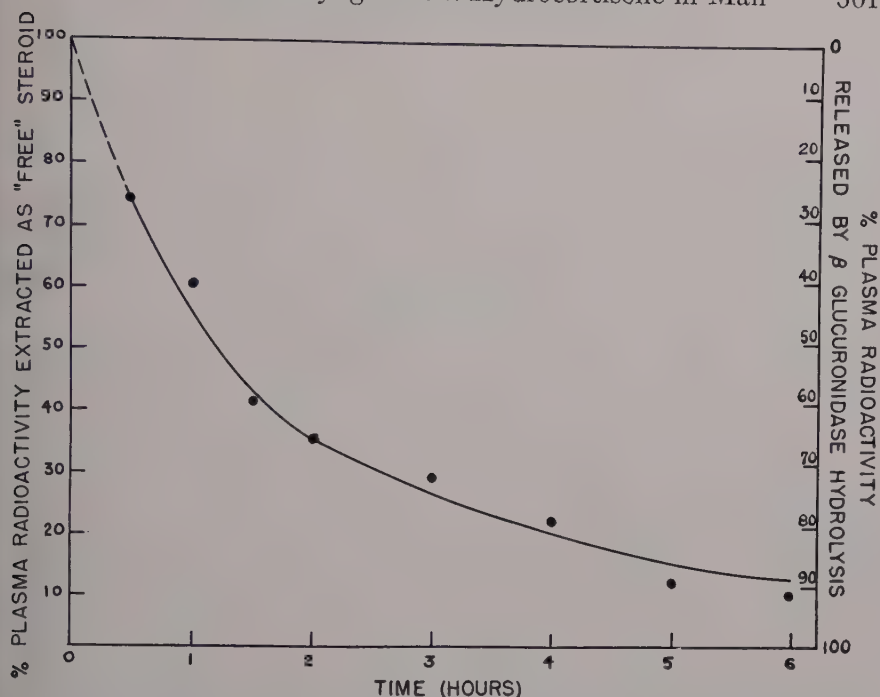


FIGURE 3. Relationship of plasma radioactivity obtained after direct extraction with dichloromethane versus plasma radioactivity as obtained after glucuronide hydrolysis and extraction with dichloromethane. The sum of the plasma radioactivity extracted directly with dichloromethane plus the plasma radioactivity in the dichloromethane extract after glucuronidase hydrolysis is assumed to represent 100 per cent of the plasma radioactivity.

slightly greater than the extracellular fluid space, and is in accord with data published by Hellman *et al.*<sup>4</sup> The interpretation that hydrocortisone is distributed only in extracellular fluid, however, is valid only if the binding of hydrocortisone in the extravascular compartments is quantitatively of the same nature as binding in the plasma.

Binding to plasma proteins does occur to an appreciable extent. At concentrations ranging from 2 to 1000 micrograms per cent, 75 per cent of the plasma hydrocortisone was found to be bound (FIGURE 4) as determined by dialysis equilibration. Separate experiments with albumin and hemoglobin have shown that these proteins are capable of binding hydrocortisone to a similar extent. Analysis of plasma hydrocortisone present in each of three fractions obtained after ultracentrifugation in 1.21 density salt solution have shown that most of the steroid eventually resides in the bottom third of the tube, the protein (mostly globulin) enriched portion.

The fact that hemoglobin is capable of binding hydrocortisone suggests that the steroid may be distributed intracellularly. Diffusion of hydrocortisone into red cells was determined by incubating with labeled hydrocortisone *in vivo* and *in vitro*. The erythrocytes took up such quantities of hydrocortisone that solution in red cell water appeared to account for only a portion of the

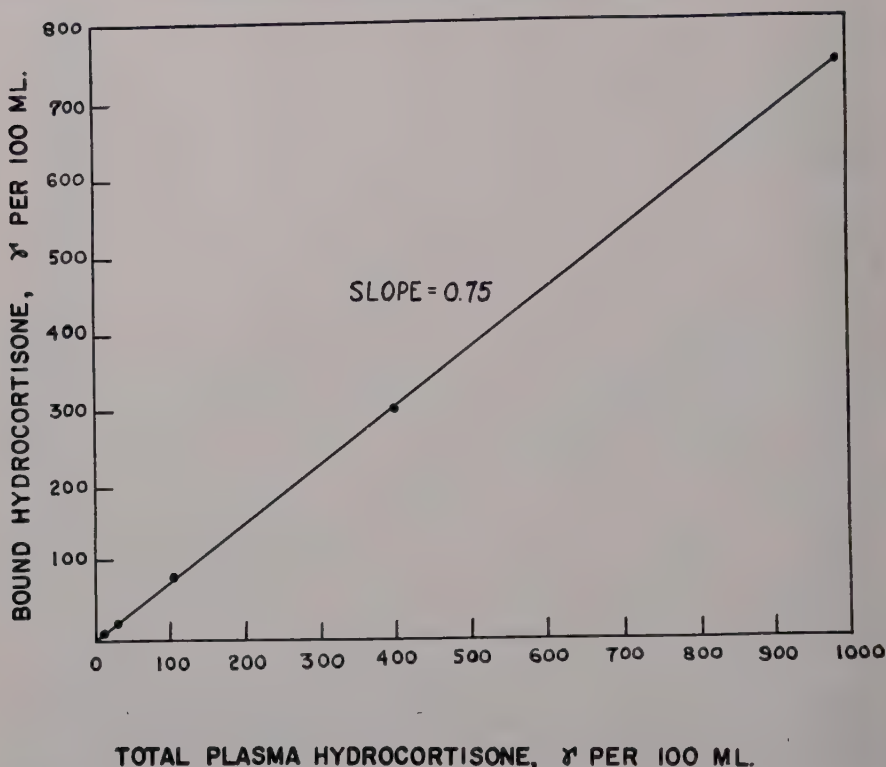


FIGURE 4. Plasma protein binding of hydrocortisone *in vitro* as determined by dialysis equilibration at 37°C for 18 hours.

total, and intracellular binding of 50 per cent of the red cell hydrocortisone content was calculated.

#### *Urinary Steroid Studies*

FIGURE 5 demonstrates the curve of cumulative excretion of radioactive metabolites of hydrocortisone in the urine following intravenous administration of a trace quantity of  $C^{14}$ -labeled hydrocortisone, and the curve of excretion following intravenous administration of labeled hydrocortisone, plus 200 mgm. of carrier steroid. More than 80 per cent appeared in the urine within the first 24 hours. An additional small amount appeared during the second and third days. By the fourth day, no radioactivity could be detected in the urine. One half of the injected dose appeared within the first three and one-half hours. Similar data have been obtained on five additional normal subjects.

The presence of identical curves of excretion for the trace and for the pharmacological doses again demonstrates that the metabolism is represented as a first order reaction, and that the rate is proportional to the concentration.

Approximately 95 per cent of the injected dose of radioactivity could be accounted for through urinary excretion in three days. Another 2 to 3 per cent



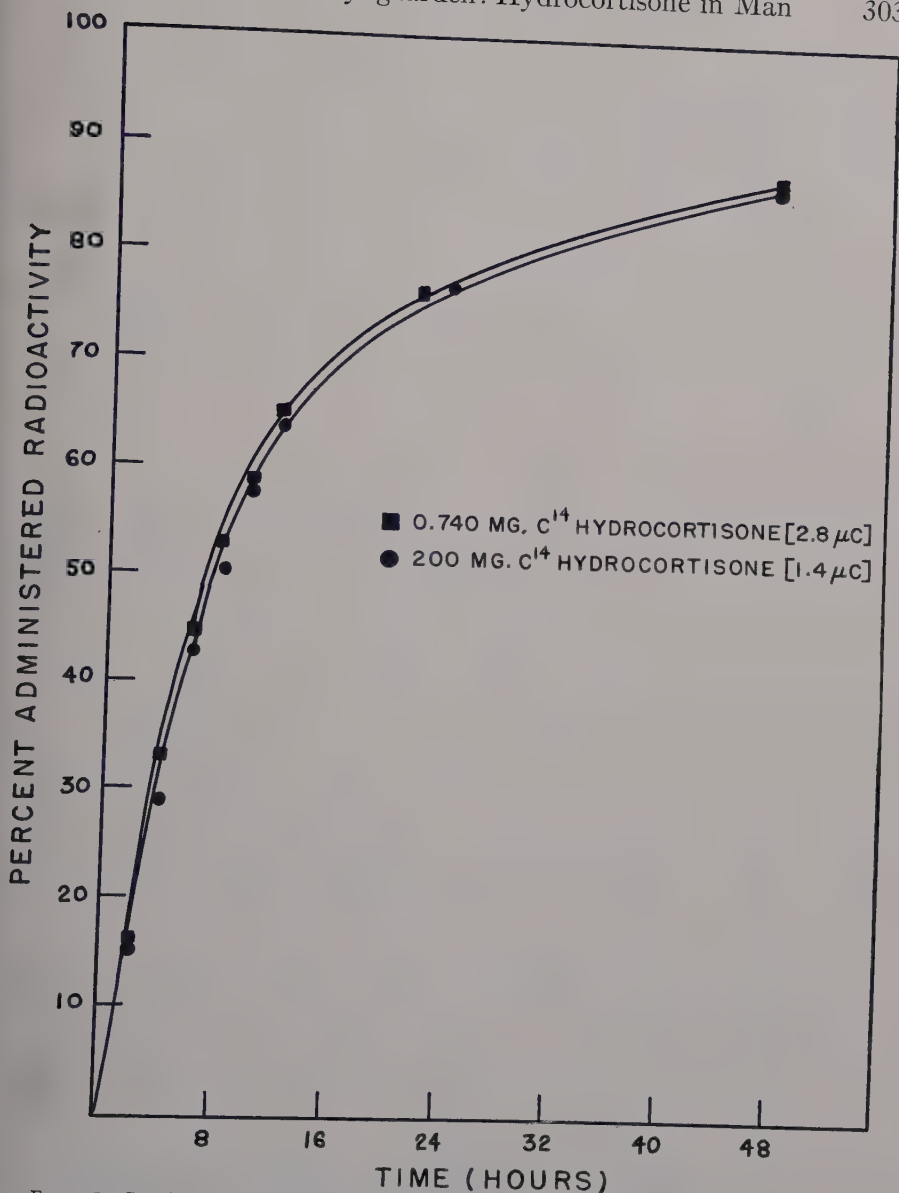


FIGURE 5. Cumulative urinary excretion of radioactivity of microgram versus milligram quantities of hydrocortisone-4-C<sup>14</sup>.

could be accounted for through fecal excretion. It has been previously reported that essentially none is excreted *via* the expired CO<sub>2</sub>,<sup>4, 5</sup> thus demonstrating that complete degradation of the steroid nucleus does not occur.

Analysis of the urinary radiometabolites has shown that about 4 per cent is

present as freely extractable material. Of this free steroid, however, only a small fraction is present as unchanged hydrocortisone—less than 1 per cent of the total administered dose.

An analysis of the radioactivity and the phenylhydrazine reacting material in the urine following intravenous administration of 200 mgm.  $C^{14}$  hydro-

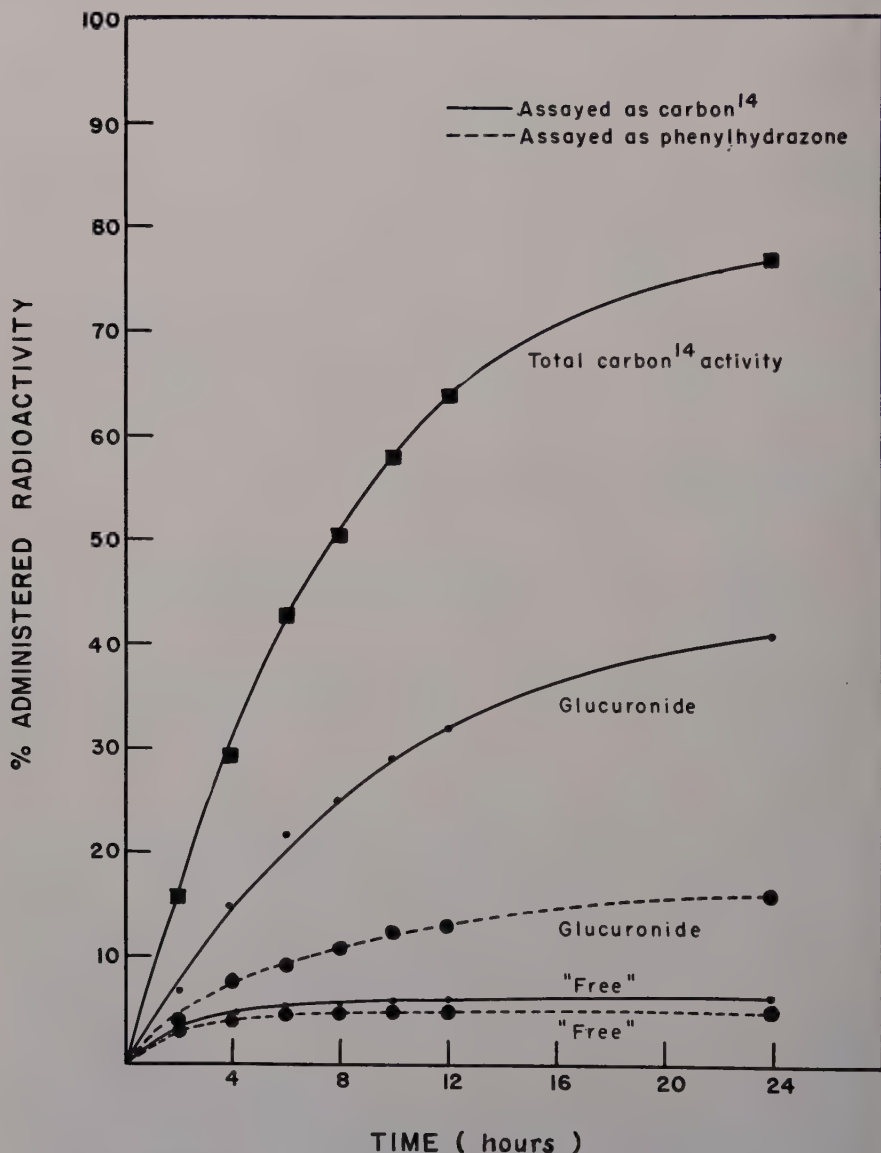


FIGURE 6. Urinary excretion of carbon<sup>14</sup> and phenylhydrazine reacting steroid following infusion of 200 mgm. and 1.4  $\mu$ c hydrocortisone-4- $C^{14}$ .

cortisone, including both direct dichloromethane extraction and dichloromethane extraction following  $\beta$ -glucuronidase hydrolysis, has shown the following (FIGURE 6): Approximately 4 per cent is present as free steroid as determined by radioactivity, and slightly less when determined with phenylhydrazine. Sixty per cent of the total radioactivity in the urine can be hydrolyzed with  $\beta$ -glucuronidase and then extracted with dichloromethane, whereas only 20 per cent of the administered dose of steroid could be recovered as phenylhydrazine reacting material after glucuronidase hydrolysis.

These two findings, the failure to hydrolyze all of the steroid conjugates present in the urine and the failure to measure all of the hydrolyzed metabolites with phenylhydrazine, make most previously published methods<sup>6, 7</sup> inadequate for quantitation of total urinary corticosteroid metabolites of hydrocortisone. Acid hydrolysis with heating releases another 15 to 20 per cent of the radioactivity.

### *Oral Absorption of Hydrocortisone*

After oral administration of a solution of either a trace quantity of labeled hydrocortisone, or isotopic hydrocortisone plus 200 mgm. of carrier, curves of urinary excretion of radiometabolites are very similar to excretion curves following intravenous administration. Only a slightly smaller percentage of the pharmacological dose was recovered in the urine. These data suggest that essentially complete absorption takes place from the gastrointestinal tract.

When a trace quantity of hydrocortisone-4- $C^{14}$  was administered to a patient with a complete biliary fistula with normal liver function, 4 per cent of the administered dose was found to be excreted *via* the bile. None was recovered in the feces, suggesting that no excretion occurred across the intestinal wall. This failure to excrete any appreciable quantity through the bile excludes the possibility of any significant degree of enterohepatic circulation.

In summarizing the pathways of the disposition of hydrocortisone, it may be said that 94 per cent can be accounted for through urinary excretion, and 2 per cent through the feces. Four per cent is excreted through the bile, leaving 2 per cent for recirculation through the enterohepatic system. Of the urinary excretion, practically the entire quantity appears as a water-soluble conjugate, mostly as the glucuronide. Of the 4 per cent that appears as free steroid, only a fraction of this, or less than 1 per cent of the administered dose, is present as unchanged hydrocortisone. Thus, the body succeeds in metabolizing over 99 per cent of the injected dose of hydrocortisone.

### *References*

1. PETERSON, R. E. Unpublished procedures.
2. SILBER, R. H. & C. C. PORTER. 1954. *J. Biol. Chem.* **210**: 923.
3. SWEAT, M. L. 1954. *Anal. Chem.* **26**: 773.
4. HELLMAN, L., H. L. BRADLOW, J. ADESMAN, D. K. FUKUSHIMA, J. L. KULP & T. F. GALLAGHER. 1954. *J. Clin. Invest.* **33**: 1106.
5. WYNGAARDEN, J. B., A. WOLFF & R. E. PETERSON. 1955. *J. Biol. Chem.* **212**: 963.
6. SANDBERG, A. A., D. H. NELSON, E. M. GLENN, F. H. TYLER & L. T. SAMUELS. 1953. *J. Clin. Endocrinol. and Metab.* **13**: 1445.
7. REDDY, W. J., D. JENKINS & G. W. THORN. 1952. *Metabolism*. **1**: 511.



# THE EFFECT OF ADRENAL STEROIDS ON RENAL MECHANISMS OF ELECTROLYTE EXCRETION\*

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The steroids elaborated by the adrenal cortex are known to affect measurably the electrolyte structure of the body. Hyperadrenocorticism produced either from an exogenous or an endogenous source results in expansion of the extracellular fluid and sodium, depletion of intracellular potassium and phosphate, and frequently an extracellular alkalosis.<sup>1, 17, 18, 21, 30, 36</sup> Hypoadrenocorticism, if severe, may be accompanied by extracellular alterations which are the opposite of those resulting from excessive amounts of adrenal steroid. The changes that have been observed consist of extracellular dehydration, acidosis, hyperkalemia, hyperphosphatemia and hyponatremia.<sup>2, 13, 15, 17, 22, 25, 26, 33, 38, 39</sup> The pathogenesis of these changes has been extensively studied<sup>1, 2, 13, 15, 17, 18, 21, 22, 25, 26, 30, 33, 36, 37, 38, 39</sup> and abundant evidence exists to indicate that cellular shifts of electrolytes and water consequent to deficiencies or excesses of adrenal steroids are responsible, in part, for the acid-base disturbances. Alterations in the renal excretion of electrolytes and water are additive to these cellular shifts in the production of the chemical and clinical abnormalities. Furthermore, the effects of adrenal cortical steroids on the urinary excretion of electrolytes may be a direct one on renal tubular mechanisms *per se* or alterations in renal excretion may be secondary to associated metabolic and cardiovascular defects which occur in conjunction with derangements of adrenal function.<sup>22</sup> The studies reported here were carried out on normal and adrenalectomized dogs and on human subjects to define the direct effects of the adrenal steroids on renal function and to evaluate the renal contributions to the production of the extracellular electrolyte and water abnormalities observed in hypo- and hyperadrenocortical states. In these experiments, the acute and chronic effects of cortisone and DOCA on the renal excretion of sodium, potassium, bicarbonate, titratable acids, and phosphate were studied. The experimental data indicate that the acute effects of cortisone are mainly an increase in the renal tubular reabsorption of sodium and a decrease in phosphate reabsorption. DOCA had an effect on sodium excretion similar to that observed with cortisone. No consistent acute effects of these steroids were observed on the renal excretion of potassium, titratable acids, or bicarbonate. With prolonged administration of cortisone, however, potassium excretion was increased and hypokalemic alkalosis was not infrequent.

## *Sodium*

The relatively small but significant moiety of sodium which is reabsorbed by virtue of the action of the adrenal steroids on the renal tubule is one of the

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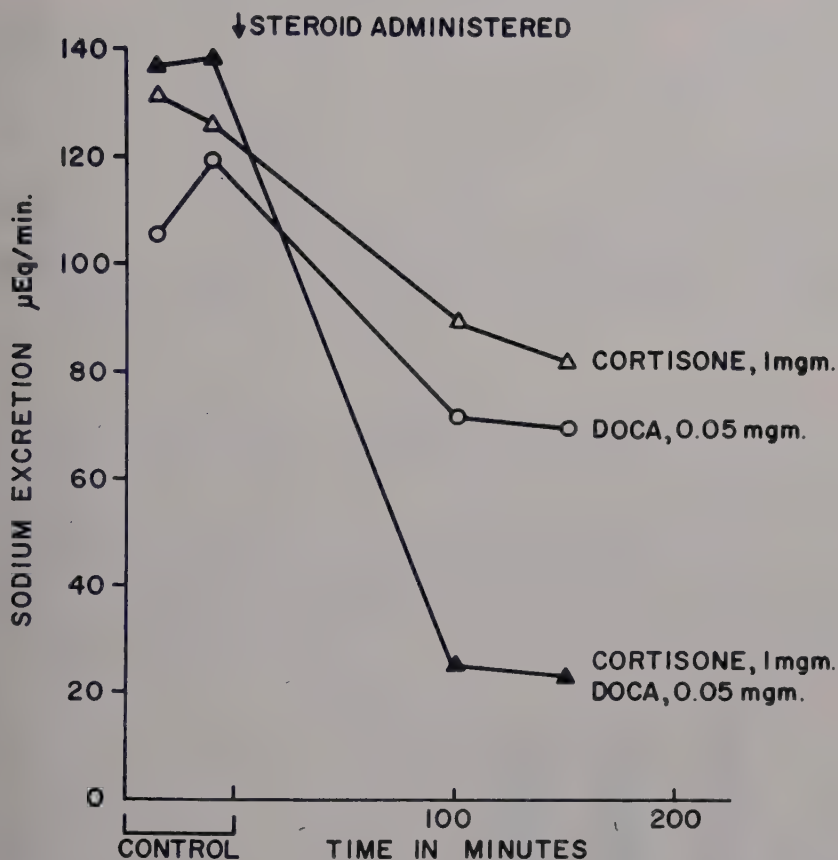


FIGURE 1. Average sodium excretion in an adrenalectomized animal previous to and following the intravenous administration of (a) cortisone (open triangles), (b) DOCA (open circles), and (c) DOCA and cortisone administered together (solid triangles).

most clearly defined renal functions which these steroids affect.<sup>2, 10, 14, 22, 25, 33, 37</sup> The observation that DOCA results in sodium retention in a wide variety of experimental circumstances is universally recognized. From more recent studies made possible by the current availability of ACTH and cortisone, it has become evident that these agents also enhance the renal tubular reabsorption of sodium in a manner qualitatively similar to that observed following the administration of DOCA.<sup>1, 18, 21, 22, 25, 30, 36</sup> Three typical experiments that were performed in an adrenally insufficient dog are shown in FIGURE 1 and illustrate (1) the acute sodium retaining effects exerted by DOCA and cortisone, (2) the relative potency of these two steroids when given alone, and (3) their additive effects when given in combination. With minimal doses of cortisone or DOCA, it was possible to quantitate the decrease in sodium excretion so that the dosage of administered steroid reduced sodium excretion by a minimal amount. When these minimal doses of cortisone and DOCA were administered simultaneously, the reduction in sodium excretion was double that seen when

each agent was injected alone. With larger doses of steroid, this additive effect could not be defined, since sodium reabsorption was already maximally altered. From the experiments illustrated here and similar experiments with larger doses of steroid, it was apparent quantitatively that DOCA was 15 to 20 times more potent than cortisone in decreasing renal sodium excretion. In none of these acute experiments was there any evidence to indicate that these two hormones were antagonistic as regards sodium excretion.

In several experiments, the simultaneous measurements of glomerular filtration rate and calculations of sodium reabsorption clearly indicated that the decrease in sodium excretion resulted from an increased tubular reabsorption and was not the result of an alteration in the filtered moiety.

### Phosphate

The negative phosphate balance occurring in hyperadrenocorticism and the hyperphosphatemia which has been observed in adrenal insufficiency have implicated an influence of adrenal cortical steroids on the renal tubular excretion of phosphate.<sup>8, 11, 16, 20, 29, 34</sup>

FIGURE 2 shows one of a series of experiments designed to study the effects of cortisone and DOCA on the maximal tubular reabsorption of phosphate (phosphate Tm). These experiments were carried out by infusing the animals with quantities of phosphate sufficient to elevate plasma levels and thus to exceed the renal reabsorptive capacity. Following an adequate stabilization

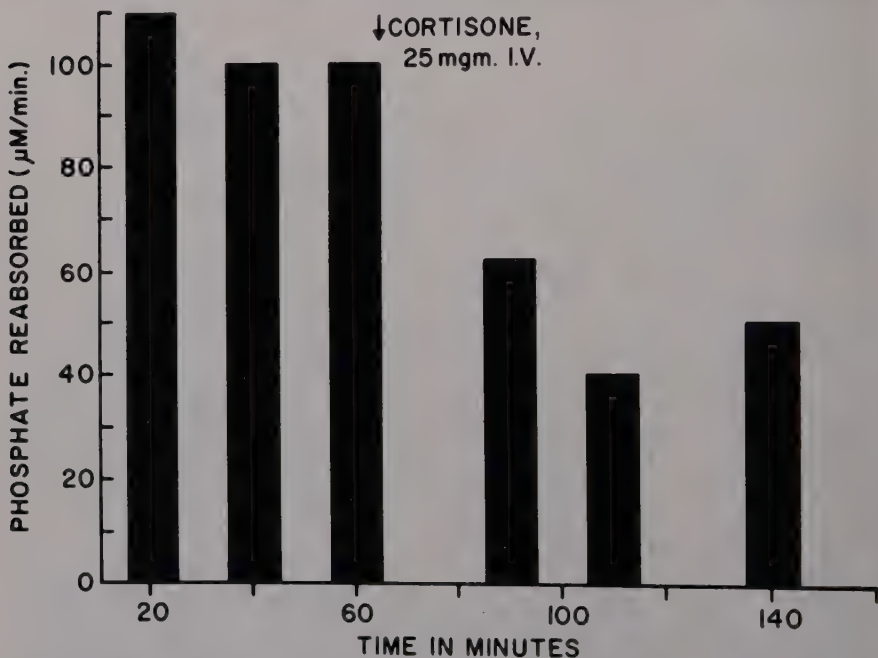


FIGURE 2. Maximal phosphate reabsorption (Phosphate Tm) in one normal dog before and after administration of 25 mgm. of cortisone intravenously.



period, the maximal tubular reabsorption of phosphate was measured previous to and following the intravenous administration of cortisone or DOCA. As illustrated in this figure, the reabsorption of phosphate was significantly decreased by the administration of cortisone. This was a consistent finding in both intact and adrenally insufficient dogs. Similar effects were not observed in the animals given DOCA.

The elevated plasma levels of phosphate that are frequently observed in severe adrenal insufficiency were, however, not clearly explicable on the basis of an enhanced phosphate reabsorption in all animals studied. Presumably, the retention of phosphate in adrenal insufficiency is also fundamentally dependent upon a decreased glomerular filtration rate and cellular shifts which have been described in hypoadrenocorticism.

### *Titrateable Acids*

The reports<sup>22, 31</sup> showing a defect in titrateable acid and ammonia secretion in adrenal insufficiency indict the adrenals in this capacity but do not clarify whether hydrogen ion secretion is directly impaired by a lack of adrenal steroid. Accordingly, several experiments were carried out to determine whether this defect was a direct one implicating the effects of cortisone on the renal tubular exchange of hydrogen for sodium. Since the amount of titrateable acid secreted into the tubular lumen is dependent, in part, upon the amount of phosphate buffer present in the glomerular filtrate, it might also be expected that alterations in titrateable acid secondary to changes in phosphate excretion would be affected by cortisone.

FIGURE 3 summarizes the average titrateable acid excretion in normal and adrenalectomized dogs both with and without hormone administration. In

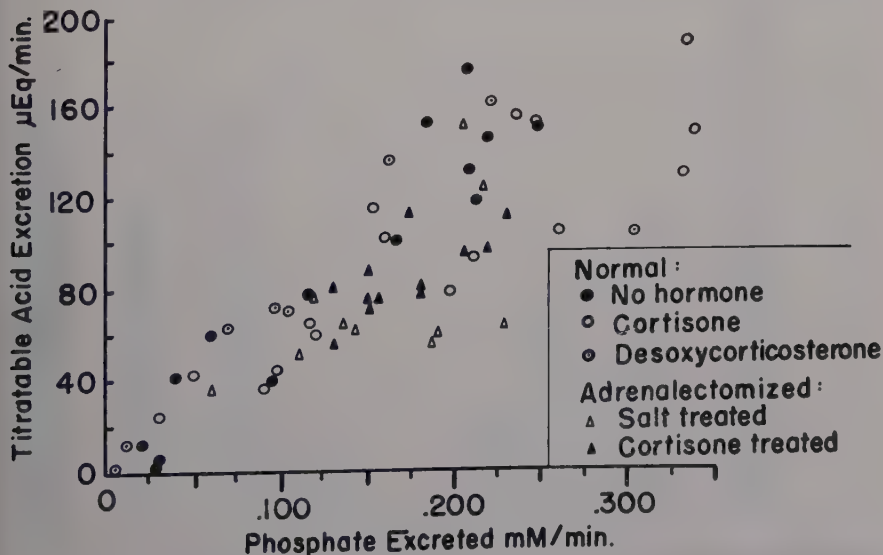


FIGURE 3. Titrateable acid excretion in relation to phosphate excretion in normal and adrenalectomized dogs both with and without hormone administration.

this figure, the titratable acid excretion per minute is plotted in relation to phosphate excretion. In none of these acute experiments was there any clear-cut evidence that cortisone or DOCA influenced titratable acid excretion when the animals were presented with a phosphate load. Rather, the alterations in titratable acid excretion that were measured appeared to be proportional to the amount of phosphate excreted in the urine in both normal and adrenalectomized dogs.

### Potassium

The renal excretion of potassium in normal and adrenalectomized dogs was extensively studied (a) during adrenal insufficiency, and (b) during periods of hormone administration.

FIGURE 4 summarizes eight of these experiments in which the renal excretion of potassium was measured in dogs given cortisone or DOCA. In these experiments, the average renal excretion of potassium was measured during a one-hour control period and during a two-hour period following the intravenous administration of steroid. As shown here, no consistent alterations in the renal excretion of potassium were measured during the two-hour interval following hormone administration. This failure to observe changes in renal excretion in acute experiments following cortisone or DOCA was also evident in normal dogs and in dogs given phosphate or bicarbonate infusions. Following steroid administration in several adrenalectomized animals, it was observed

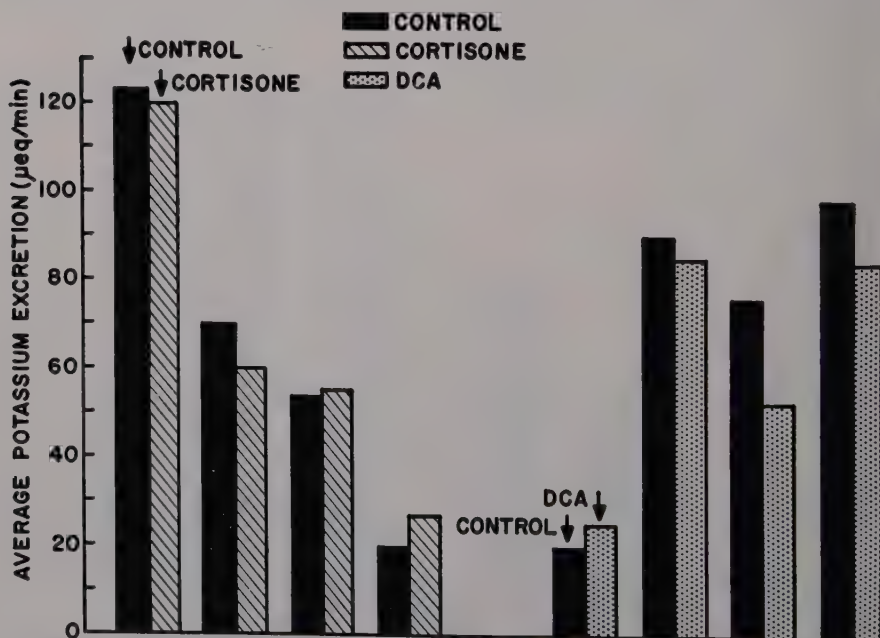


FIGURE 4. Average potassium excretion in eight experiments during a one-hour control period (solid bars) and during a two-hour interval following the administration of cortisone (cross-hatched bars) or DCA (dotted bars).

that the elevated plasma potassium level often decreased to normal despite a decreased urinary excretion of this ion. These studies emphasize the importance of cellular shifts of potassium as a fundamental basis for the hyperkalemia occurring in adrenal insufficiency and imply that the kidney is not solely responsible.<sup>2, 21, 22, 25, 26</sup>

In contrast to these acute findings, it has often been observed by us and by others<sup>7, 35</sup> that chronic administration of adrenal hormone may result in an increase in renal potassium excretion and, if sufficiently prolonged, may result in negative potassium balance with hypokalemia. These experiments imply that renal excretion of potassium may not be directly influenced by cortisone or DOCA but that a negative potassium balance following prolonged steroid administration may be related, in part, to abnormal protein and carbohydrate metabolism, cellular shifts of potassium or other metabolic defects.

### Bicarbonate

The chronic administration of the adrenal steroids or ACTH commonly results in a plasma alkalosis and the coincident excretion of an acid urine. This is illustrated in TABLE 1, which also shows the cumulative potassium balance in a patient who was receiving cortisone. In this patient, the measured potassium depletion resulted in a plasma alkalosis that was reversed by the administration of sufficient quantities of potassium even in the presence of continued cortisone therapy. This occurrence of a plasma alkalosis with an acid urine is not unique in hyperadrenocorticism, but it may be seen in a variety of clinical situations in which depletion of intracellular potassium is the common denominator.<sup>9, 23, 27, 28</sup> Evidence presented elsewhere indicates that potassium deficiency alkalosis is observed in a variety of clinical situations and is related both to cellular shifts of electrolytes<sup>5, 6, 12, 19</sup> and enhanced renal tubular reabsorption of bicarbonate.<sup>3, 4, 24, 28</sup> The coexistence of potassium depletion and alkalosis in chronic hyperadrenocorticism implies a similar basis for its origin. The coincident potassium depletion, however, does not definitely preclude a direct effect of the adrenal steroids on the renal tubular reabsorption of bicarbonate bound base. Therefore, a series of experiments were carried out on normal dogs to study the effects of cortisone acutely on renal

TABLE 1  
CUMULATIVE POTASSIUM BALANCE AND PLASMA ELECTROLYTES IN A PATIENT TREATED WITH CORTISONE

Potassium (Cumulative balance)		Plasma			
Intake	Output	Sodium	Potassium	Chloride	Bicarbonate
<i>Total mEq. for 10 days</i>		<i>mEq./L</i>			
1105	1470	134	3.2	94	37
Potassium supplements					
1595	1290	132	4.2	107	26



TABLE 2  
RENAL TUBULAR REABSORPTION OF BICARBONATE BEFORE AND AFTER ADMINISTRATION OF  
CORTISONE

Time minutes	Plasma		Glom. filt. Rate	Urine bicarbonate		
	Bicarbonate	Potassium		Filt.	Excr.	Reabsorbed
	mEq./L		cc/min.	mEq./min.		mEq./100 cc. G. F.
-150	Infuse creatinine, 1% NaHCO <sub>3</sub> , G/W @ 5 cc./min.					
20	31.4	3.03	119	3.74	.361	2.84
40	31.4	—	115	3.61	.366	2.82
60	31.6	3.05	112	3.54	.348	2.85
Cortisone 25 mg. i.v.						
100	31.3	3.05	124	3.88	.364	2.83
140	30.6	—	120	3.67	.324	2.79
160	30.1	3.06	130	3.91	.346	2.74
180	30.0	3.05	126	3.78	.337	2.73

bicarbonate reabsorption. One of these experiments is shown in TABLE 2. In the animals studied by us, sodium bicarbonate was infused throughout the experimental period in order to insure an adequate plasma level and thus exceed the renal reabsorptive capacity. The effects of cortisone were then quantitated in regard to the amount of bicarbonate reabsorbed per 100 cc. of glomerular filtrate. The data shown here illustrate the failure of cortisone to alter bicarbonate reabsorption acutely. In none of these experiments was bicarbonate reabsorption elevated to more than 2.85 milliequivalents per 100 cc. of glomerular filtrate, a change considered by us to be of doubtful significance. From this we have assumed that the plasma alkalosis accompanying chronic administration of adrenal steroids does not result from a direct effect of the steroids on renal bicarbonate reabsorption but is more fundamentally related to the alterations occasioned by potassium depletion. The well-known reversal or prevention of the plasma alkalosis by adequate potassium supplementation furnishes additional evidence for this assumption.

#### *Glomerular Filtration Rate and Renal Blood Flow*

It should be emphasized that changes in the renal excretion of electrolytes following DOCA or cortisone have been observed even in the absence of alterations in glomerular filtration and with minimal changes in renal blood flow. During the acute experiments reported here, we were unable to measure significant changes in these renal functions in adrenalectomized dogs following cortisone or DOCA. Only with prolonged administration of hormone and with adequate maintenance therapy did alterations in the glomerular filtration rate or renal blood flow become apparent. Therefore, we have presumed that the acute alterations in electrolyte excretion occasioned by steroid administration are the result of a direct action of cortisone or DOCA on the tubular reabsorptive mechanisms.

ELECTROLYTE	RENAL EXCRETION FOLLOWING CORTISONE	
	ACUTE	CHRONIC
SODIUM	↓	↓
CHLORIDE	↓	↓
POTASSIUM	+ -	↑
BICARBONATE	+ -	
PHOSPHATE	↑	↑
TITRATABLE ACID	+ -	+ -

FIGURE 5. Alterations in the renal excretion of electrolytes with acute and chronic administration of cortisone.

### Summary

FIGURE 5 summarizes the acute and chronic effects of cortisone on renal electrolyte excretion under the conditions imposed by our experiments. Sodium and chloride retention following cortisone administration appeared to be a constant feature in both normal and adrenalectomized dogs. Phosphate Tm was found to be decreased following cortisone administration, although a similar alteration in phosphate reabsorption was not evident with DOCA administration. No consistent effect of cortisone *per se* on titratable acid was measured during an acute experimental period. The failure to observe consistent changes in potassium excretion or bicarbonate reabsorption following intravenous cortisone acutely was in marked contrast to the negative potassium balance, increased bicarbonate reabsorption, and the plasma alkalosis observed with chronic steroid administration. Evidence has been presented which indicates intracellular potassium depletion as a causative factor in the enhanced bicarbonate reabsorption and plasma alkalosis that frequently accompany prolonged steroid administration.

### References

1. BAEHR, G. & L. J. SOFFER. 1950. Bull. N. Y. Acad. Med. **26**: 229.
2. BARNETT, H. L. & H. McNAMARA. 1949. J. Clin. Invest. **28**: 1498.
3. BERLINER, R. W., T. J. KENNEDY & J. ORLOFF. 1951. Am. J. Med. **11**: 274.
4. BERLINER, R. W. 1954. Ann. Rev. Physiol. **16**: 269.

5. COOKE, R. E., W. E. SEGAR, C. S. REED, D. D. ETZWILER, M. VITA, S. BRUSILOV & D. C. DARROW. 1954. *Am. J. Med.* **17**: 180.
6. COOKE, R. E., W. E. SEGAR, D. B. CHEEK, F. E. COVILLE & D. C. DARROW. 1952. *J. Clin. Invest.* **31**: 798.
7. DARROW, D. C. & H. C. MILLER. 1942. *J. Clin. Invest.* **21**: 601.
8. ELIEL, L. P., L. HELLMAN, O. H. PEARSON & B. KATZ. 1951. *Proc. 2nd Clin. ACTH Conf.* Blakiston Press. **1**: 196.
9. ELIEL, L. P., O. H. PEARSON & R. W. RAWSON. 1950. *New Engl. J. Med.* **243**: 471, 518.
10. FERREBEE, J. W., C. RAGAN, D. ATCHLEY & R. F. LOEB. 1939. *J. Am. Med. Assoc.* **113**: 1725.
11. FREEMAN, S., J. FERSHING, C. C. WANG & L. C. SMITH. 1950. *Proc. 1st. Clin. ACTH Conf.* Blakiston Press. **1**: 509.
12. GARDNER, L. J., E. A. MACLACHLAN & H. BERMAN. 1952-1953. *J. Gen. Physiol.* **36**: 153.
13. GUADINO, M. & M. F. LEVITT. 1949. *J. Clin. Invest.* **28**: 1487.
14. HARROP, G. A., W. M. NICHOLSON & M. STRAUSS. 1936. *J. Exptl. Med.* **64**: 233.
15. HARROP, G. A., L. J. SOFFER, R. ELLSWORTH & J. H. TRESCHER. 1933. *J. Exptl. Med.* **58**: 17.
16. INGBAR, S. H., E. H. KASS, C. H. BURNETT, A. S. RELMAN, B. A. BURROWS & J. H. SISSON. 1951. *Proc. 2nd Clin. ACTH Conf.* Blakiston Press. **1**: 130.
17. LOEB, R. F. 1941-1942. *Harvey Lectures*. : 100.
18. MCEWEN, C., J. J. BUNION, J. S. BALDWIN, A. J. KUTTNER, S. B. APPEL & A. J. KALTMAN. 1950. *Bull. N. Y. Acad. Med.* **26**: 212.
19. ORLOFF, J., T. J. KENNEDY & R. W. BERLINER. 1953. *J. Clin. Invest.* **32**: 538.
20. PEARSON, O. H., L. P. ELIEL & R. W. RAWSON. 1950. *Proc. 1st Clin. ATCH Conf.* Blakiston Press. : 318.
21. PERERA, G. A., K. S. PINES, H. B. HAMILTON & K. VISLOCKY. 1949. *Am. J. Med.* **56**: 7.
22. PITTS, R. F. 1951. *Adrenal Cortex*. E. P. Ralli, Ed. *Trans. 3rd Conf. Josiah Macy, Jr. Found., New York, N. Y.* : 11-52.
23. RANDALL, H. T., D. V. HABIF, J. S. LOCKWOOD & S. C. WERNER. 1949. *Surgery*. **26**: 341.
24. RELMAN, A. S., B. ETSTEN & W. B. SCHWARTZ. 1953. *J. Clin. Invest.* **32**: 972.
25. ROBERTS, K. E. & R. F. PITTS. 1952. *Endocrinology*. **50**: 51.
26. ROBERTS, K. E. 1952. *Proc. Soc. Exptl. Biol. Med.* **79**: 32.
27. ROBERTS, K. E., M. G. MAGIDA & R. F. PITTS. 1953. *Am. J. Physiol.* **172**: 42.
28. ROBERTS, K. E., H. T. RANDALL, H. L. SANDERS & M. HOOD. *J. Clin. Invest.* In press.
29. ROBERTS, K. E. & R. F. PITTS. 1953. *Endocrinology*. **52**: 324.
30. RUSSELL, J. A. 1950. *Bull. N. Y. Acad. Med.* **26**: 229.
31. SARTORIUS, O. W., D. CALHOUN & R. F. PITTS. 1953. *Endocrinology*. **52**: 256.
32. SELDEN, D. W., L. G. WELT & J. CORT. 1951. *J. Clin. Invest.* **30**: 6.
33. SMITH, H. W. 1951. *The Kidney: Structure and Function in Health and Disease*. Chap. 12. Oxford Univ. Press. Oxford, England.
34. SOFFER, L. J., J. L. GABRILOVE & J. W. JAILER. 1950. *J. Clin. Endocrinol.* **10**: 594.
35. SPRAGUE, R. G. 1951. *Am. J. Med.* **10**: 567.
36. SPRAGUE, R. G., M. H. POWER, H. L. MASON, A. ALBERT, R. MATHIESON, P. S. HENCH, E. C. KENDALL, C. H. SLOCUMB & H. F. POLLEY. 1950. *Arch. Internal Med.* **85**: 199.
37. THORN, G. W., L. L. ENGELL & H. EISENBERG. 1938. *J. Exptl. Med.* **68**: 161.
38. WATERHOUSE, C. & E. H. KEUTMANN. 1948. *J. Clin. Invest.* **27**: 372.
39. WHITE, H. L., P. HEINBECKER & D. ROLF. 1947. *Am. J. Physiol.* **149**: 417.



# COMPARISON OF THE METABOLIC EFFECTS OF CORTISONE AND HYDROCORTISONE IN MAN\*

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The metabolic effects of cortisone and hydrocortisone in man are qualitatively similar.<sup>1</sup> This is evidenced by the ability of both hormones to produce such changes as sodium chloride and water retention, potassium diuresis, increased excretion of nitrogen and uric acid, and a rise in the level of blood and urinary glucose.

There are, however, *quantitative* differences in the metabolic actions of cortisone and hydrocortisone which vary according to the route of administration and state of esterification of the compounds. Early studies in animals by Ingle *et al.*<sup>2, 3</sup> and Olson and his associates<sup>4</sup> demonstrated that hydrocortisone administered by intramuscular or subcutaneous injection possessed greater metabolic activity than cortisone. The criteria used were work performance of adrenalectomized rats, glycogen deposition activity in adrenalectomized rats, and diabetogenic effect in force-fed normal rats. That the difference in potency between hydrocortisone and cortisone was not due to a difference in either rate of absorption or speed of inactivation was unequivocally demonstrated by the studies of Ingle *et al.*,<sup>5</sup> in which the hormones were administered by continuous intravenous infusion and assayed by the muscle work test. Hydrocortisone was found to be approximately twice as effective as cortisone.

Comparative studies in man, with the free alcohol of cortisone and hydrocortisone administered intravenously, have confirmed the superior potency of hydrocortisone.<sup>6</sup> This is illustrated in FIGURE 1, which shows the eosinopenic effect of hydrocortisone to be greater than that of cortisone.

In addition to the demonstration of its greater potency when administered intravenously, hydrocortisone in the form of either the acetate or the free alcohol has been shown by Conn<sup>7</sup> and Thorn *et al.*<sup>8</sup> to be more effective than cortisone acetate when the compounds are given *by mouth*.

In contrast to these findings, Salassa and his associates,<sup>9</sup> Conn,<sup>7</sup> and others<sup>1</sup> have shown that when the hormones are given in the form of the acetate ester by intramuscular injection the metabolic effects of hydrocortisone are slower in onset, less intense, and more prolonged than those of cortisone. This difference would appear to be due to a slower rate of absorption of hydrocortisone acetate from the intramuscular depot. Thus, while hydrocortisone acetate by intramuscular injection is of no value in situations where a rapid and intense hormone effect is required, it may prove useful in the long-term treatment of patients with virilism due to bilateral adrenal hyperplasia where satisfactory adrenal inhibition may be achieved by injection of the hormone every one to three weeks.<sup>10</sup>

\* The cortisone and hydrocortisone used in this study were kindly provided by Doctors H. F. Hailman and C. J. O'Donovan of the Upjohn Company, Kalamazoo, Mich., and by Doctor Elmer Alpert of Merck and Co., Inc., Rahway, N.J. The fluorohydrocortisone was obtained through the generosity of Doctors Josef Fried and H. A. Strade of the Squibb Institute for Medical Research, New Brunswick, N.J.

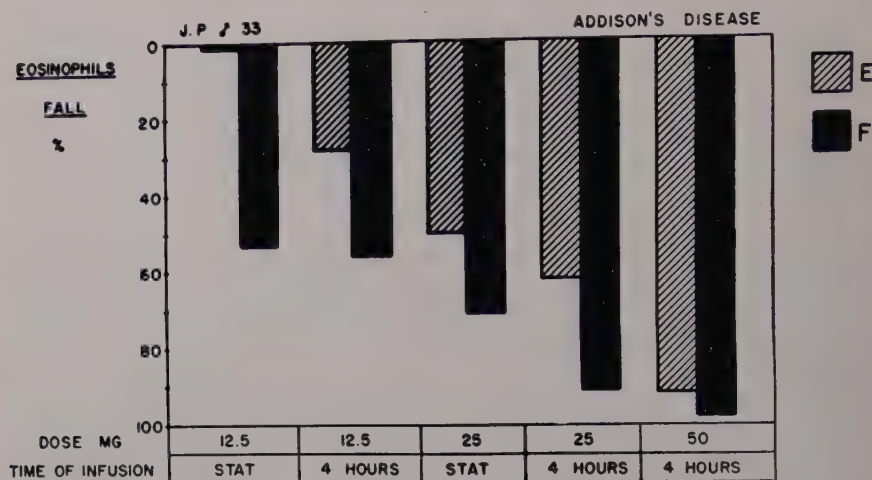


FIGURE 1. Response of the blood eosinophils to the intravenous administration of hydrocortisone (compound F) and cortisone (compound E) in a 33-year-old man with Addison's disease.

In contrast to its acetate ester, the free alcohol of hydrocortisone is as effective and rapid in its action when administered by intramuscular injection as when given by mouth.<sup>7</sup> The greater potency and rapidity of action of hydrocortisone-free alcohol, as compared to its acetate ester, when both compounds are given intramuscularly, are shown in FIGURE 2.

Both cortisone and hydrocortisone are considerably more active when administered (as the free alcohol) by intravenous infusion than when given in divided doses by mouth.<sup>6</sup> This is demonstrated for hydrocortisone in FIGURE 3. The pronounced electrolyte regulating activity of intravenously administered hydrocortisone is particularly well demonstrated in the type of study illustrated in FIGURE 4. Sodium retention occurred within two hours after the beginning of the hydrocortisone infusion and reached a maximum by the end of six hours. It is to be noted that the urinary sodium excretion fell from an initial level of 6 mEq. to 0.5 mEq. per hour. It is possible that the increased effectiveness of hydrocortisone or cortisone when given intravenously is due in part to the destructive action of gastric secretion on the orally administered hormone, a finding recently reported by Sandberg and his associates.<sup>11</sup>

Finally, it has been demonstrated that hydrocortisone is considerably more effective than cortisone when administered topically in inflammatory disease of the skin,<sup>12</sup> the eye,<sup>13</sup> and the joints.<sup>14</sup>

The reason for the greater metabolic activity of hydrocortisone is not clear. It is not known whether hydrocortisone more effectively penetrates the cell membrane than cortisone or whether the hormone itself is the active substance, hydrocortisone being more potent than cortisone at the final site of action. It has been suggested, from the finding of the relative inefficacy of topically-administered cortisone, that this substance, to be therapeutically active, must be transformed to hydrocortisone or that hydrocortisone is converted more

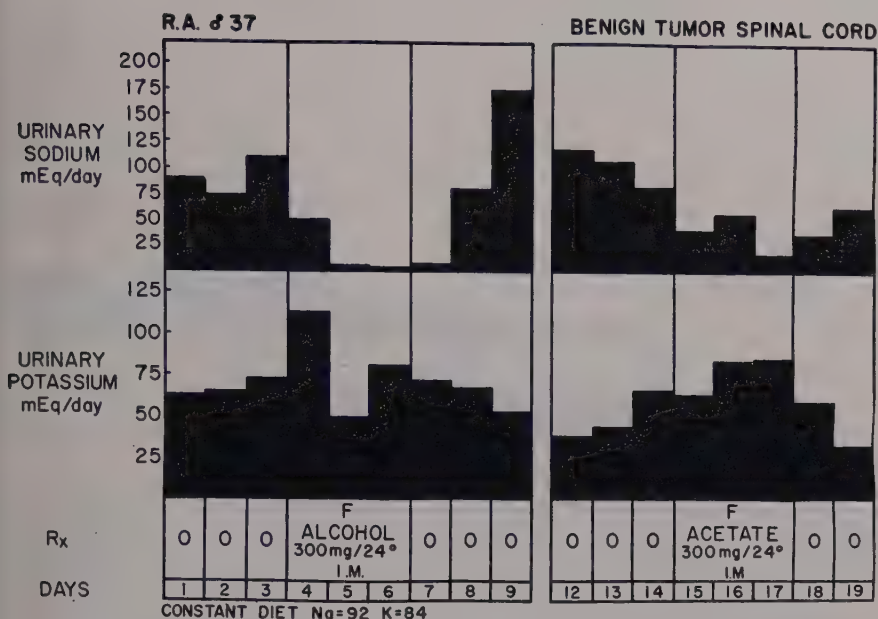


FIGURE 2. Effect of the intramuscular administration of the free alcohol and the acetate of hydrocortisone (compound F) on sodium and potassium excretion in a 37-year-old man with intact adrenals.

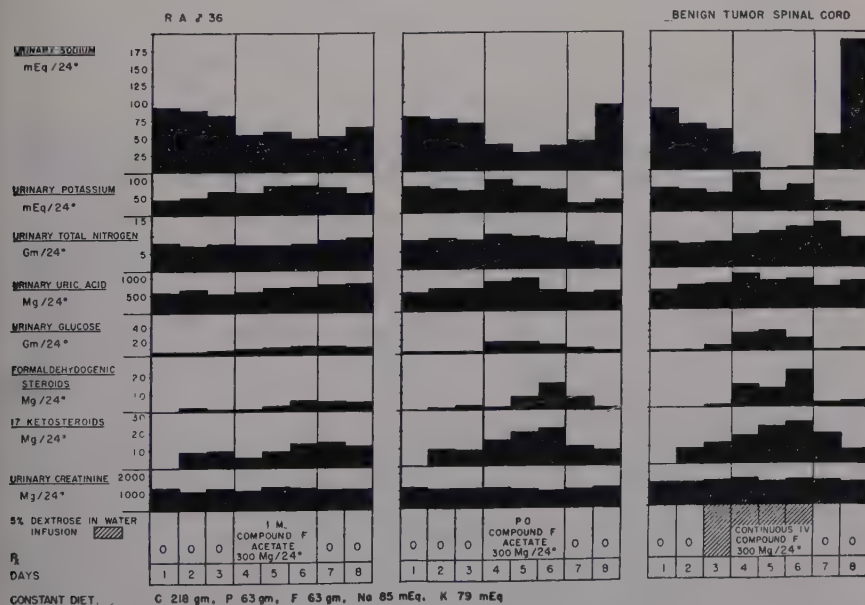


FIGURE 3. Metabolic effects of hydrocortisone (compound F) administered by intramuscular, oral, and intravenous routes to a 36-year-old man with intact adrenals. When given by mouth or by intramuscular injection, the hormone was administered in divided doses, 75 mg. every six hours.

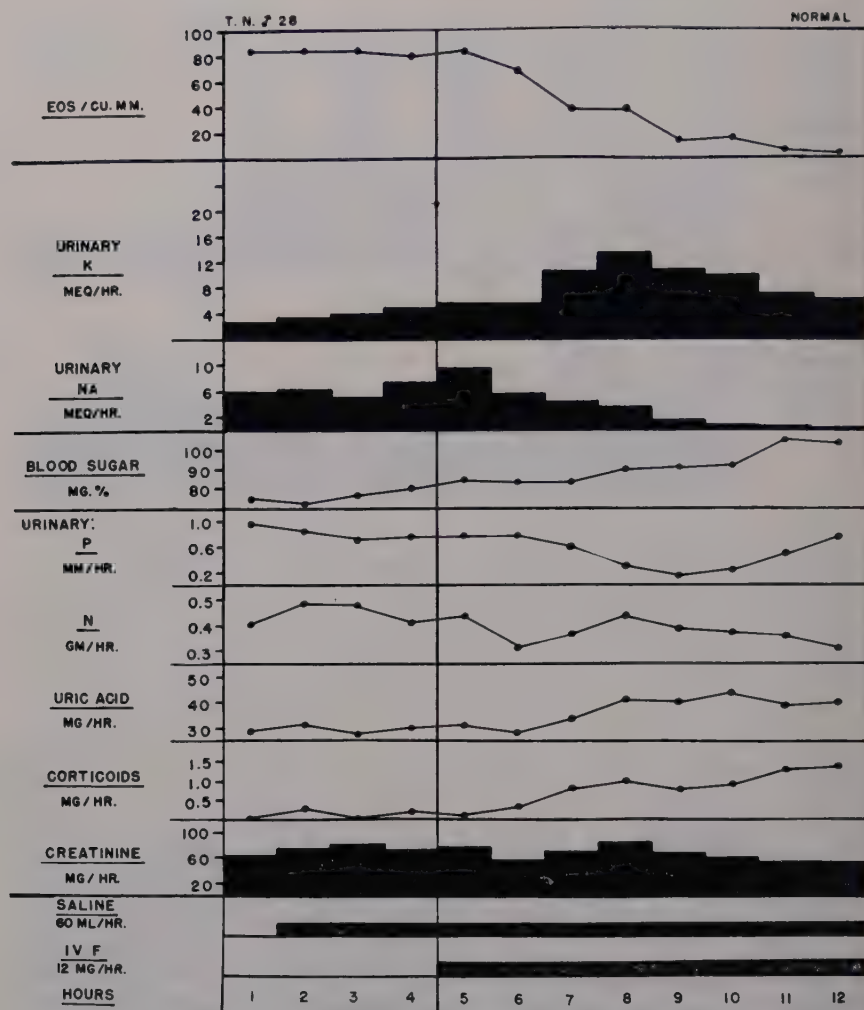


FIGURE 4. Metabolic effects of intravenously administered hydrocortisone (compound F) in a 28-year-old normal male subject. The subject was in the fasting state throughout the experiment. (This figure appeared in "Pharmacological aspects of adrenocortical steroids and ACTH" by G. W. Thorn *et al.* 1953. *New Engl. J. Med.* 248: 141. It is reprinted with permission of the *New England Journal of Medicine*.)

efficiently than cortisone to a hypothetical active metabolite.<sup>15</sup> With regard to the latter possibility, it is of interest that there is another respect in which hydrocortisone is more potent than cortisone; namely, its ability to serve as a precursor of 17-ketosteroids and androgens. Evidence for this has come from studies of the 17-ketosteroid and androgen excretion following the intravenous or oral administration of the hormones to orchidectomized, adrenalectomized patients.<sup>16</sup> The results of one such study are shown in FIGURE 5. It is not being suggested, of course, that one of the 17-ketosteroid metabolites of cortisone and hydrocortisone is the hypothetical active substance. It is only



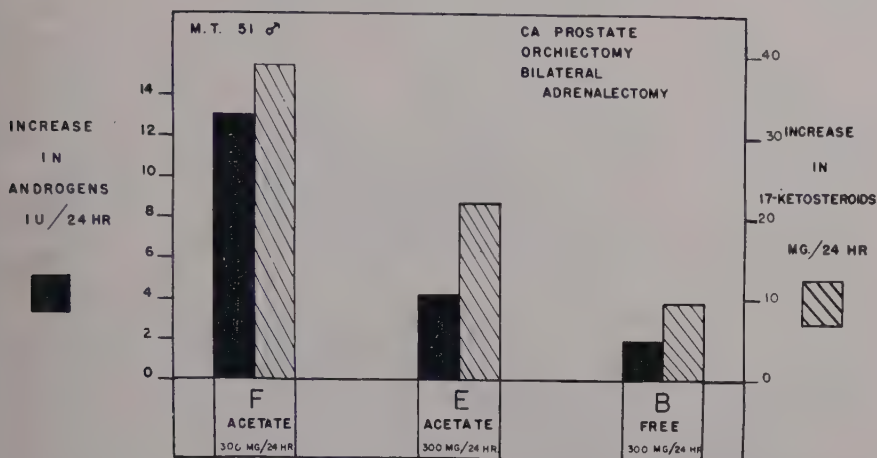


FIGURE 5. Increases in urinary excretion of androgens and 17-ketosteroids (above the levels observed during period of no treatment) produced by daily oral doses of 300 mg. of hydrocortisone acetate, of cortisone acetate, and of corticosterone in a 51-year-old adrenalectomized, orchidectomized patient. (This figure appeared in "Effect of adrenocortical steroids on androgen excretion by adrenalectomized orchidectomized men" by P. L. Munson *et al.*, 1954. *J. Clin Endocrinol. & Metab.* 14: 495. It is reprinted with permission of the *Journal of Clinical Endocrinology and Metabolism*.)

being pointed out that it is possible to demonstrate a difference, quantitative at least, in the metabolism of the two hormones.

The remainder of this paper will be confined to a consideration of certain aspects of but one of the many effects of cortisone and hydrocortisone in man; namely, their effect on electrolyte metabolism.<sup>17</sup>

It has been noted<sup>1</sup> that the influence of these hormones on electrolyte excretion is somewhat more variable than that of either desoxycorticosterone or corticosterone. Not infrequently, it has been observed that cortisone and hydrocortisone may produce an increase, rather than a decrease, in sodium excretion, particularly during the early period of hormone administration. As an approach to the study of this problem, observations have been made on renal dynamics and electrolyte excretion in Addisonian subjects.<sup>18</sup> The studies were carried out during control periods and during periods of intravenous infusion of hydrocortisone. These patients were on a constant diet and were maintained on oral cortisone and on monthly injections of desoxycorticosterone trimethyl acetate. On the day prior to each study, the cortisone was discontinued. On the day of study, infusions of 5 per cent dextrose in water containing inulin and p-aminohippuric acid were begun at 8 A.M. and continued at the rate of 100 ml. per hour until 12 noon on the following day. Four hours after the start of the infusion on the days of hormone administration, hydrocortisone free alcohol was added to the intravenous solution and continued at the rate of 1 or 10 mg. per hour for a period of 24 hours. Urines were collected in two-hour periods and blood samples were taken at appropriate intervals for measurement of inulin and PAH clearance and electrolyte filtration and excretion. The results of one such study are illustrated in FIGURE 6. It can be seen that, when hydrocortisone was infused at the rate of 1 mg. per hour, there was a

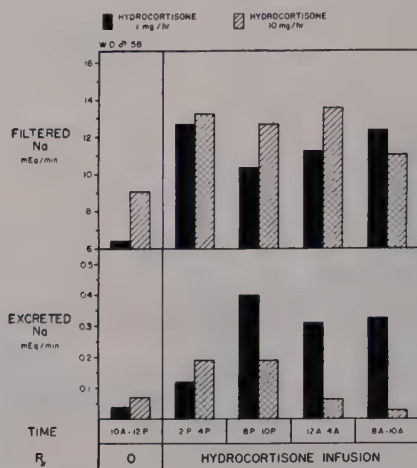


FIGURE 6. Effect of continuous intravenous hydrocortisone on renal filtration and excretion of sodium in a 58-year-old man with Addison's disease.

sustained increase in sodium filtration associated with a sustained sodium diuresis. With the administration of hydrocortisone at the rate of 10 mg. per hour, an initial sodium diuresis occurred in association with a rise in sodium filtration. Subsequently, sodium retention was observed despite a continuing elevation of filtration. It would appear that, in the Addisonian subject receiving the smaller dose of hydrocortisone, the sodium diuresis was the result of a relatively greater increase in glomerular filtration than in tubular reabsorption, while the subsequent sodium retention with the larger dose of hormone resulted from further enhancement of tubular reabsorption.

The reverse of the effects just described and the profound electrolyte changes which occur as the result of a deficiency of adrenal cortical hormones are illustrated in the following study of exchangeable body sodium and potassium<sup>19</sup> in a 37-year-old man with Addison's disease (FIGURE 7).

During the three months prior to admission, the patient had shown progressive weight loss, anorexia, nausea, vomiting, dehydration and hypotension. On admission (April 2, 1954) the serum sodium concentration was low: 121 mEq. per liter; the serum potassium concentration high: 8.0 mEq. per liter. Despite these values, the levels of exchangeable body sodium and potassium, as measured in mEq./kg., were within the normal range. Moreover, the patient was not found to be in negative sodium balance. Therapy was started two days after admission with 6.25 mg. of fluorohydrocortisone daily by mouth. Two weeks after admission (April 16, 1954), the patient felt very much better in spite of excessive fluid and salt retention, manifested by edema, pleural effusion, cardiac enlargement, a weight gain of 12 kilograms, and a rise above normal of the exchangeable body sodium. By this time, the exchangeable body potassium had fallen well below the normal range. Thereafter, the dose of fluorohydrocortisone was reduced, first to 1 mg., then to 0.5 mg., and finally to 0.25 mg. given as a single oral dose daily. By June 3, 1954, the patient felt

	April 2, 1954	April 16, 1954	June 3, 1954
WEIGHT kg.	54	66	68
$\text{Na}_E$ meq	2461	3955	3224
$\text{Na}_E$ meq./kg	45 (40.9-44.7)	60	47
$\text{K}_E$ mcg	2558	2305	3531
$\text{K}_E$ meq./kg.	47 (47.0-54.5)	35	52
$\text{Na}_E/\text{K}_E$	0.96 (.84-1.06)	1.71	0.91
CLINICAL CONDITION	ADDISONIAN CRISIS	EXCESSIVE FLUID RETENTION	MARKED IMPROVE- MENT
Rx	0	FLUORO F Ac 6.25 MG./24° P.O.	FLUORO F Ac 0.25 MG./24° P.O.
	WG. 6° 37		ADDISON'S DISEASE

FIGURE 7. Exchangeable body sodium ( $\text{Na}_E$ ) and exchangeable body potassium ( $\text{K}_E$ ) in a 37-year-old man with Addison's disease. The values given in brackets in the left-hand column indicate the normal range for  $\text{Na}_E$ ,  $\text{K}_E$ , and  $\text{Na}_E/\text{K}_E$  ratio.

in good health and had returned to full-time work as a machinist. He had lost the excess fluid but had gained a further 2 kilograms in weight above the level of April 16. The serum sodium and potassium concentrations were normal, and the levels of exchangeable body sodium and potassium, which had been abnormal at the time of excessive fluid and salt retention, had returned to normal. The value for exchangeable body sodium expressed in milliequivalents per kilogram was, in fact, little different from that obtained when the patient was in Addisonian crisis.

In this patient, there is little doubt that, at least in the early stages of development of adrenal insufficiency, there was a loss of sodium from the extracellular fluid *via* the kidney. However, the finding of normal levels of exchangeable body sodium and potassium in association with low serum sodium and high serum potassium concentrations and evidence of diminished plasma volume suggest that with the progression of adrenal insufficiency there was a gradual increase in intracellular sodium and decrease in intracellular potassium. On the basis of such changes, it is possible to account for the failure to observe a negative sodium balance at the time of Addisonian crisis by the following mechanism. The diminished capacity of the renal tubules to reabsorb sodium could be compensated for by reduced glomerular filtration due to dehydration and hypotension and by a decreased sodium concentration in the glomerular filtrate due in part to a shift of sodium from the extracellular fluid into the cells.

In the study of the action of adrenal cortical hormones such as cortisone and hydrocortisone on electrolyte metabolism, one must, therefore, consider an

effect not only on renal function but also on the distribution of sodium and potassium between the intracellular and extracellular compartments.

### Conclusions

(1) Hydrocortisone free alcohol is more potent than cortisone whether given intravenously, orally or by intramuscular injection.

(2) When the hormones are given in the form of the acetate ester by intramuscular injection, the metabolic effects of hydrocortisone are slower in onset, less intense, and more prolonged than those of cortisone.

(3) Cortisone and hydrocortisone free alcohol are more effective when administered by continuous intravenous infusion than they are when given in divided doses by mouth. The increased potency with regard to electrolyte regulating activity is particularly striking.

(4) The variable effects of cortisone and hydrocortisone on renal sodium excretion would appear to be due in part, at least, to variable degrees of enhancement of glomerular filtration and tubular reabsorption of the ion.

(5) Further evidence has been provided to indicate that the influence of adrenal cortical hormones on electrolyte metabolism is exerted not only through their effect on renal function but also through an effect, direct or indirect, on the distribution of sodium and potassium between the extracellular and intracellular compartments.

### References

1. THORN, G. W., D. JENKINS, J. C. LAIDLAW, F. C. GOETZ, J. F. DINGMAN, W. L. ARONS, D. H. P. STREETEN & B. H. McCracken. 1953. Pharmacological aspects of adrenocortical steroids and ACTH in man. *New Engl. J. Med.* **248**: 232, 284, 323, 369, 414, 632.
2. INGLE, D. J. & M. H. KUIZENGA. 1945. Relative potency of some adrenal cortical steroids in muscle-work test. *Endocrinology*. **36**: 218.
3. INGLE, D. J. 1940. Work performance of adrenalectomized rats treated with corticosterone and chemically related compounds. *Endocrinology*. **26**: 472.
4. OLSON, R. E., S. A. THAYER & L. J. KOPP. 1944. Glycogenic activity of certain crystalline steroids of adrenal cortex when administered singly and with cortical extract to fasted, normal and adrenalectomized rats. *Endocrinology*. **35**: 464.
5. INGLE, D. J., J. E. NEZAMIS & E. H. MORLEY. 1951. Work performance of adrenalectomized rats given cortisone and 17-hydroxycorticosterone by continuous intravenous injection. *Proc. Soc. Exptl. Biol. Med.* **78**: 79.
6. JENKINS, D., J. A. GARCIA-REYES, B. H. McCracken & G. W. THORN. Unpublished observations.
7. CONN, J. W. Discussion of SPRAGUE, R. G. 1952. Clinical use of adrenal cortical hormones and ACTH. *Trans. 3rd. Conf. on the Adrenal Cortex*. E. P. Ralli, Ed. Josiah Macy, Jr. Found., New York, N. Y.
8. THORN, G. W., A. E. RENOLD, D. L. WILSON, T. F. FRAWLEY, D. JENKINS, J. GARCIA-REYES & P. H. FORSHAM. 1951. Clinical studies on the activity of orally administered cortisone. *New Engl. J. Med.* **245**: 549.
9. SALASSA, R. M., M. H. POWER, H. L. MASON & R. G. SPRAGUE. 1952. Comparative metabolic effects of intramuscular use of cortisone acetate and 17-hydroxycorticosterone (Compound F) acetate. *J. Clin. Invest.* **31**: 658.
10. SEGALOFF, A., D. GORDON, A. FLORES & B. N. HORWITT. The treatment of adrenal hyperplasia with virilism by infrequent injections of hydrocortisone acetate. *J. Lab. Clin. Med.* In press.
11. SANDBERG, A. A., K. EIK-NES, D. H. NELSON, J. G. PALMER, G. E. CARTWRIGHT & M. M. WINTROBE. 1954. Adrenocortical function and metabolism of 17-hydroxycorticosteroids in pernicious anemia. *New Engl. J. Med.* **251**: 169.
12. GOLDMAN, L. & I. BASKETT. 1952 (Dec.). Compound F in dermatology. Presented



at the Acad. Dermatol. and Syphilol. of the College of Medicine of the Univ. of Cincinnati, Cincinnati, Ohio.

13. SMITH, R. W., JR. & E. H. STEFFENSEN. 1951. ACTH and cortisone in treatment of ocular disease. *New Engl. J. Med.* **245**: 972, 1007.
14. HOLLANDER, J. L. 1951. Local effects of Compound F (hydrocortisone) injected into joints. *Bull. Rheumatic Diseases.* **2**: 3.
15. PINCUS, G. 1952. Some basic hormone problems. *J. Clin. Endocrinol. & Metab.* **12**: 1187.
16. MUNSON, P. L., F. C. GOETZ, J. C. LAIDLAW, J. H. HARRISON & G. W. THORN. 1954. Effect of adrenocortical steroids on androgen excretion by adrenalectomized orchidec-tomized men. *J. Clin. Endocrinol. & Metab.* **14**: 495.
17. THORN, G. W., J. C. LAIDLAW & A. GOLDFIEN. 1955. Studies on the sodium-retaining effect of adrenal cortical steroids. *Colloquia on Endocrinology.* G. E. W. Wolsten-holme, Ed. J. and A. Churchill. London, England. In press.
18. FINKENSTAEDT, J. T., J. F. DINGMAN, D. JENKINS, J. C. LAIDLAW & J. P. MERRILL. 1954. The effect of intravenous hydrocortisone and corticosterone on the diurnal rhythm in renal function and electrolyte equilibria in normal and Addisonian subjects. *J. Clin. Invest.* **33**: 933.
19. ARONS, W. L., R. J. VANDERLINDE & A. K. SOLOMON. 1954. The simultaneous meas-urement of exchangeable body sodium and potassium utilizing ion exchange chromatog-raphy. *J. Clin. Invest.* **33**: 1001.

# ADRENAL STEROIDS AND THE SECRETION OF DIGESTIVE ENZYMES\*

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In his early (1855) description of the syndrome of adrenocortical deficiency in man, Thomas Addison<sup>1</sup> drew attention to the severe gastrointestinal disturbance which may occur. More recent experimental studies further demonstrated that the adrenal cortex exerts an important supportive influence on the function of the alimentary tract. The absorption of fat,<sup>2</sup> sodium chloride,<sup>3</sup> and glucose<sup>4</sup> is reduced by adrenalectomy. Maintenance of the animal on a salt regimen restores the absorptive rate for glucose<sup>4</sup> to normal but fails to do so with respect to fat.<sup>2</sup>

Interference with secretory processes is indicated also by the reduction in weight of the gastrointestinal mucosa which follows adrenalectomy.<sup>5</sup> The volume of gastric secretion is reduced in adrenocortical deficiency<sup>6, 7, 8</sup> and treatment with desoxycorticosterone acetate and cortisone acetate has proved only partially effective in restoring this deficiency.<sup>9</sup> Interference with the secretion of hydrochloric acid occurs also. The reduction in the amount of free acid in the stomachs of patients with Addison's disease is well known. In experimental animals, also, the secretion of acid is reduced by adrenalectomy.<sup>6, 7</sup> The volume and acidity of gastric secretion and the reduced intestinal absorption of fat<sup>2</sup> are restored to normal in the adrenalectomized animal by the administration of Upjohn's Cortin.

This presentation is concerned with the role of adrenocortical hormones in the regulation of the cytology of and the secretion of enzymes by various serous cells associated with the digestive tract. Tuerkischer and Wertheimer<sup>7</sup> were the first to demonstrate that the capacity of rats to produce rennin and pepsin is impaired by adrenalectomy, a condition which was corrected by administration of Upjohn adrenocortical extract. More recently, Gray and his collaborators<sup>10</sup> have demonstrated that an increased secretion of pepsin and excretion of uropepsin in man results from treatment with corticotropin and cortisone. Little is known concerning the relationship of the adrenal cortex to the activity of other zymogenic cells.

The following zymogenic cells will be discussed: the gastric chief cells, and the epithelial cells of the parotid and pancreatic acini. Our work and that of others indicates that the hormonal background which regulates these cells is much broader than that exerted by the adrenal cortex alone. We shall attempt to assess the role of adrenocortical hormones in so far as the presently available fragmentary evidence will permit.

Hypophysectomy induces significant cytological changes in all of the types of zymogenic cells listed above. Gastric chief cells become smaller and are depleted of their pepsinogen granules and cytoplasmic ribonucleic acid.<sup>11</sup> Concurrently, there is a reduction in the capacity of these cells to secrete pepsin

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during a six-hour period after pyloric ligation. This suppression is detectable three days after pituitary ablation, and is reduced by 80 per cent at seven days.

As has been demonstrated by others<sup>12, 13, 14</sup> and confirmed by us<sup>15</sup> in the rat, hypophysectomy results in a marked loss in weight of the pancreas. Cytologically, the acini became smaller and the intracellular content of zymogenic granules is reduced. The parotid gland presents a similarly striking atrophy following pituitary ablation.<sup>15</sup>

In order to ascertain the hormonal pathway by which the pituitary gland affects the zymogenic cells, it was necessary to study the effect of excision of other endocrine glands which are under the control of the anterior hypophysis. If pituitary control is exerted entirely through the adrenal cortex, adrenalectomy should induce changes comparable with those which occur after hypophysectomy. Evidence concerning this point is available with respect to the gastric chief cells. Cytologically, adrenalectomy induces an atrophy which, generally, is not as severe as that elicited by hypophysectomy. At three days after adrenalectomy, the mean peptic activity per ml. of gastric juice is  $86.2$  hemoglobin proteolytic\* units ( $\text{PU}^{\text{Hb}} \times 10^{-4}$ ) and the total activity of the sample is  $731 \times 10^{-4}$  in animals maintained on saline.<sup>16</sup> Three days after hypophysectomy, however, the comparable figures are  $80.0 \text{ PU}^{\text{Hb}} \times 10^{-4}$  and  $325 \text{ PU}^{\text{Hb}} \times 10^{-4}$ , respectively. The difference in the total activity of the juice in the two experiments is accounted for by the much greater drop in the volume of gastric juice secreted after hypophysectomy as compared with that after adrenalectomy. Maintenance of adrenalectomized rats on water instead of saline does not alter significantly the effect of the operation. Gonadectomy is without effect on the cytology or secretory capacity of the chief cells. Thyroidectomy does not modify the cytology, or concentration of peptic activity in the gastric juice. It does reduce the volume of juice produced and, thereby, the total peptic activity. A combination of adrenalectomy, thyroidectomy, and gonadectomy in the same animal induces a cytological and secretory change in the chief cells which, at seven days after the operation, is comparable with the effects induced by hypophysectomy.<sup>16</sup> Thus, it appears that pituitary control of the chief cells is exerted primarily through the adrenal cortex with the thyroid being also involved.

Studies concerned with the restoration of the atrophied zymogenic cells of hypophysectomized rats by replacement therapy also support the conclusion that adrenocortical hormones play an important role in their regulation. The daily administration of hydrocortisone† to hypophysectomized rats beginning on the day of operation partially prevents the atrophy of the gastric chief cells which would be expected to occur during the succeeding seven days.<sup>16</sup> Similarly, the daily injection of cortisone acetate to hypophysectomized rats beginning 9 to 13 days after the operation restores to normal the concentration of peptic activity of the gastric juice but, because of a continued depression of the volume of juice produced, the total peptic activity remains at a subnormal level.<sup>16</sup> Thus, it is apparent that the adrenal cortex is involved in an important

\* Hemoglobin proteolytic units of peptic activity = mEq. of tyrosine released from hemoglobin by pepsin of the gastric juice during one minute of digestion at  $35.5^{\circ}\text{C}$ .

† We extend our appreciation to Doctor Elmer Alpert of Merck and Co., Inc., Rahway, N. J., for generous supplies of hydrocortisone and cortisone acetate.

way in the depression of chief cell activity which ensues after hypophysectomy. Since ablation experiments indicate that the thyroid might be involved also, replacement experiments were carried out in which thyroxine was administered. At a 3- $\mu$ g. daily dosage given for seven days, no cytological change was observed. The capacity to secrete pepsin was not ascertained. The effect of higher doses given for longer periods of time remains to be investigated. Somatotropin modified the cytology but not the functional capacity of the cells.<sup>15</sup> Thus, replacement studies confirm the evidence obtained from ablation experiments and indicate that adrenocortical steroids of the 11-oxygenated type are important in controlling the secretion of pepsin by chief cells.

Less information is available concerning the action of adrenocortical hormones on the pancreas and parotid gland of hypophysectomized animals. Administration of cortisone acetate (0.5 mg. daily for 7 days, beginning 14 days after hypophysectomy) induces an increase in weight of the pancreas, although it is not restored to normal. This effect is of particular significance because the hormonal treatment causes a concurrent decrease in body weight.<sup>16</sup> The weight of the parotid glands in these hypophysectomized rats was not increased by therapy with cortisone acetate.

In conclusion, it has been demonstrated that hypophysectomy causes severe atrophy of the zymogenic cells of three organs; *i.e.*, stomach, pancreas, and parotid gland. Therapy with cortisone and/or hydrocortisone restores partially the structure and/or function of the gastric chief cells and acinar epithelium of the pancreas. This restoration has not been effected in the parotid. These findings are far from conclusive because varied doses and periods of therapy, as well as other adrenocortical steroids must be studied before the role of the adrenal cortex in the regulation of enzyme secretion will be entirely clear. As of the moment, this function appears to be an important one.

### References

1. ADDISON, T. 1855. On the constitutional and local effects of disease of the supra-renal capsules. Highley. London, England.
2. BAVETTA, L., L. HALLMAN, H. J. DEUEL, JR. & P. O. GREELEY. 1941. The effect of adrenalectomy on fat absorption. *Am. J. Physiol.* **134**: 619.
3. CLARK, W. G. 1939. Effect of adrenalectomy upon intestinal absorption of sodium chloride. *Proc. Soc. Exptl. Biol. Med.* **40**: 468.
4. ALTHAUSEN, T. L., E. M. ANDERSON & M. STOCKHOLM. 1939. Effect of adrenalectomy and of NaCl on intestinal absorption of dextrose. *Proc. Soc. Exptl. Biol. Med.* **40**: 342.
5. HAEGER, K., D. JACOBSON & G. KAHLSON. 1953. Atrophy of the gastro-intestinal mucosa following hypophysectomy or adrenalectomy. *Acta Physiol. Scand. Suppl.* **111**: 161.
6. HAROUTINIAN, L. M. & H. L. SEGAL. 1952. The Shay rat as an assay animal for anti-ulcer factors: IV. The effect of adrenalectomy and nephrectomy. *Gastroenterology.* **21**: 556.
7. TUERKISCHER, E. & E. WERTHEIMER. 1945. Adrenalectomy and gastric secretion. *J. Endocrinology.* **4**: 143.
8. MADDEN, R. J. & H. H. RAMSBURG. 1951. Adrenalectomy in the Shay rat. *Gastroenterology.* **18**: 128.
9. MADDEN, R. J. & H. H. RAMSBURG. 1951. Gastric secretion in the adrenalectomized rat. *Endocrinology.* **49**: 82.
10. GRAY, S. J., C. RAMSEY, R. W. REIFENSTEIN & J. A. BENSON, JR. 1953. The significance of hormonal factors in the pathogenesis of peptic ulcer. *Gastroenterology.* **25**: 156.
11. BAKER, B. L. & G. D. ABRAMS. 1954. Effect of hypophysectomy on the cytology of the



fundic glands of the stomach and on the secretion of pepsin. *Am. J. Physiol.* **177**: 409.

12. KOSTER, S. 1930. Experimentelle Untersuchung der Hypophysenfunktion beim Hunde. *Pflüger's Arch.* **224**: 212.
13. GRIFFITHS, M. 1941. The influence of anterior pituitary extracts on the insulin content of the pancreas of hypophysectomized rats. *J. Physiol.* **100**: 104.
14. KINASH, B., I. MACDOUGALL, M. A. EVANS, F. E. BRYANS & R. E. HAIST. 1953. Effects of anterior pituitary extracts and of growth hormone preparations on the islets of Langerhans and the pancreas. *Diabetes.* **2**: 112.
15. BAKER, B. L. & G. D. ABRAMS. Growth hormone (somatotropin) and the glands of the digestive system. *In Proc. Intern. Symp. on The Hypophyseal Growth Hormone, Nature and Actions.* Blakiston. In press.
16. ABRAMS, G. D. & B. L. BAKER. 1954. The cytology and secretory activity of gastric zymogenic cells after ablation of ductless glands. *Gastroenterology.* **27**: 462.
17. GLASS, G. B. JERZY, B. L. PUGH & S. WOLF. 1951. A new modification of the hemoglobin technic for the determination of pepsin in gastric juice adapted for a wide range of values. *Rev. Gastroenterology.* **18**: 670.

# THE USE OF STEROIDS AS ANTI-INFLAMMATORY AGENTS\*

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## *Introduction*

Inflammation is a response to an injurious stimulus composed of a series of interrelated progressive phenomena which occur in the following order: localized cellular destruction; increased capillary permeability and edema formation; stickiness of the endothelium; margination of leukocytes; and finally, entrance of nonautochthonous cells into the injured tissue. Each of these events presents unique problems which should be evaluated with respect to the possibility of enhancing or moderating the inflammatory response to different stimuli. Thus, it is possible that any one of these phenomena may predominate under certain conditions of stimulation. Regardless of quantitative variations in the basic pattern, however, certain fundamental changes characterize all acute inflammation.

Since the authors' first suggestion<sup>1</sup> that adrenocortical hormones exert antiphlogistic influence *sui generis*, it has been demonstrated by numerous authors that each of these separate phases of acute inflammation may be moderated by treatment with certain adrenocortical hormones, particularly cortisone and hydrocortisone (reviewed by Dougherty<sup>2</sup>). Particular attention is paid here to the local tissue reaction which occurs in the loose connective tissue which is the site of inflammation.

The investigations presented here include studies of the antiphlogistic influence of several steroid hormones on the inflammatory response induced by a variety of stimuli. Further, a group of closely related steroid hormones have been assayed for their comparative anti-inflammatory and anticortisone activity. Since this method of assay allows a quantitative estimation of potency ratios, the structure activity relationships of these hormones may be deduced. Possible mechanisms by which anti-inflammatory hormones exert their effects are also considered. It is emphasized that no attempt has been made to include all of the work or points of view of other investigators. Several reviews on this subject are available for this purpose (Dougherty<sup>2</sup>; Selye *et al.*<sup>3</sup>).

## *Method for Analyzing Localized Acute Inflammatory Response*

An analysis of the antiphlogistic action of adrenal cortical steroids was made using different inflaming substances. This was done, utilizing a method by which the changes in cellular morphology, fluctuations in cell population, and alterations in tissue structure could be evaluated in the sequence in which the phenomena of inflammation occur. The antiphlogistic test described here is based on fluctuations in numbers of autochthonous and nonautochthonous cells in localized areas of subcutaneous tissue six hours after topical injection of inflaming substances.

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Nine hundred intact and adrenalectomized CBA mice, 10 to 16 weeks of age, of both sexes, were used in these experiments. All animals were kept at a constant external temperature and were fed *ad libitum* on dog chow (Purina), vitamin supplement, and water. Adrenalectomy was performed by way of the usual bilateral posterior approach under ether anesthesia two hours prior to the start of experiment. The abdomen of all animals was shaved with an electric clipper 6 to 24 hours prior to treatment. Inflammatory responses were induced with a variety of stimuli given subcutaneously in a volume generally ranging from 0.2 to 0.25 cc. This volume was chosen to insure a sufficient amount of the phlogistic substances to produce an area of adequate spread for the subsequent biopsy. The site of injection was marked by circling the area with a wax pencil dipped in xylol. The inflamed loose connective tissue was studied at various time intervals after inducing the tissue reaction.

This same method was used in assay experiments in which animals were given steroid hormones in addition to the standard dose of inflammation-inducing substance. The volume of injected material and the site of injection were the same as for control animals. The details for the preparation of the connective tissue spreads are the same as those described by Kolouch.<sup>4</sup> At various time intervals following treatment, the skin of the animals was cut along the wax pencil mark and, with the aid of a curved ocular scissors, thin sheets of areolar tissue were excised. Such sheets were spread quickly on microscopic slides, dried with an electric fan, and stained with May-Grünwald-Giemsa in a manner identical to the method used for blood films. At least five spreads of loose connective tissue were removed from the inflamed area of each animal. With the aid of a reticule (Net Micrometer 5 mm.<sup>2</sup> divided into 100  $\frac{1}{2}$  mm. squares) the autochthonous and nonautochthonous cells were counted on at least 5 mm.<sup>2</sup> (1 mm.<sup>2</sup>/spread). The reticules are glass discs with finely etched scales. They are inserted into the microscope ocular and are used for measuring or counting objects observed through the microscope. Before use, these reticules were calibrated with respect to an object of known size. This had to be repeated for different objectives and ocular magnifications, and also when used in different microscopes. A stage micrometer 2 mm. long, divided into 200 parts, was used for calibration. For general counting, a 95-power oil immersion objective together with an ocular of 10 times magnification, was used. By this method, a counting factor ranging from 13.5 to 15.6 was established, which means that from 13.5 to 15.6 reticule fields have to be counted to cover 1 mm.<sup>2</sup> of tissue (depending on the microscopic arrangement used).

The cells are counted by a tally in a manner identical to leukocyte counts in a counting chamber. To avoid counting a cell twice, those on an outside line are counted only when on the top and left field margin, or in the right or bottom lines. The following cells were considered in the counts: fibroblasts, polymorphonuclear leukocytes, lymphocytes, histiocytes, eosinophils, and mast cells. Macrophages were classified as "histiocytes" since the former term is functional rather than morphological. Other occasional cells, such as plasma cells, were tallied in the total count, but were not considered in the differential tabulation. From the counts assembled for each control and experimental

study, the percentage distribution of connective tissue cells, together with the standard error for each dosage level, was computed and compared by the method of least squares.

### *Allergic Inflammation*

Approximately 150 mice of both sexes were used in experiments on allergic inflammation. The animals were sensitized with 0.7 cc. and 0.5 cc. of horse serum (Difco) given intraperitoneally two days apart. Nineteen days after the second sensitizing dose, the local inflammation induced by antigen administration into the loose connective tissue of the abdomen was studied. A uniform challenge dose of 0.0005 cc. of horse serum per 20 grams of mouse in a total volume of 0.25 cc. saline was given to all animals. This amount of horse serum was selected because it had been established previously as the anaphylactic LD<sub>50</sub> when given intravenously to similarly sensitized adrenalectomized animals.<sup>5</sup> The mice were adrenalectomized and challenged three to four hours later. Sensitized animals were then divided into two experimental groups.

To animals of group 1, cortisone acetate (1 mg./20 gm. mouse) was given intraperitoneally two hours after adrenalectomy and two hours before the administration of horse serum. The second group of animals received antigen alone four hours after operation. Three control groups were used in these experiments: (1) intact nonsensitized mice; (2) intact sensitized animals; and (3) adrenalectomized sensitized animals. From five to seven mice were used in all instances at each of the time intervals, at which subcutaneous spreads were taken. The connective tissue spread method outlined above was employed. In those groups in which no topical inflammatory reaction resulted, the spreads were taken from a comparable abdominal site.

Sensitization alone did not produce alterations of any significance in the morphology or number of cells in the subcutaneous tissue. The local inflammatory response in sensitized animals challenged with the antigen was qualitatively the same in intact and adrenalectomized mice. The classical phenomenon of fibroblastic destruction (fibroblastolysis) and the invasion of non-autochthonous cells occurred. The adrenalectomized sensitized animals given the topical challenging dose of horse serum had a significantly greater degree of inflammation than that found in the intact sensitized group.

Cortisone (1 mgm./20 g. mouse) given to sensitized adrenalectomized animals two hours prior to administration of the challenging dose markedly decreased the destruction of fibroblasts and the invasion of both polymorphonuclear leukocytes and macrophages.<sup>1</sup> This amount of cortisone did not completely suppress the inflammation, but greatly diminished it and shortened the period of its duration.

We may summarize this aspect of the antiphlogistic function of cortisone and hydrocortisone in allergic inflammation by quoting from our earlier studies. It was concluded that "adrenal cortical secretions unquestionably have a marked antiphlogistic action on the inflammation produced by allergy as well as that which follows other phlogogenic stimuli."<sup>1</sup> Further, as we pointed out in 1950, "adrenal cortical secretion and exogenous cortisone inhibit allergic



inflammation through an antiphlogistic action *sui generis* rather than by interfering with the antigen-antibody union."<sup>1</sup>

### Histamine Induced Inflammation

For the study of the antiphlogistic action of cortisone and other related adrenal steroids in histamine injected animals, the same method as given above was employed. Male CBA mice, 10 to 16 weeks old, were used in all experiments. Immediately following adrenalectomy, control animals were given a standard amount (.25 cc.) of a solution of 0.2 mg. histamine diphosphate in .85 per cent sodium chloride. This dose of histamine, used as a standard inflaming stimulus, was injected into the subcutaneous tissue of the abdomen. This same procedure was used in preparing tissue from experimental animals which were given graded amounts of the hormones. The hormones were added to the standard phlogistic dose of histamine diphosphate used in the control experiments. The mixture of hormone and histamine was given subcutaneously to the experimental animals in the same volume of saline solution as that used in control studies.

Destruction of fibroblasts, the first alteration seen in the inflamed area, was observed within less than two hours in the histamine treated control animals (FIGURE 1). Cortisone and compound F almost completely prevented this inflammatory phenomenon and were effective over a wide dosage range (FIGURE 1). The number of fibroblasts showing lysis and nuclear degeneration was decreased in a linear fashion when cortisone was given in the dosage range from .125  $\gamma$  to .5  $\gamma$ . On the other hand, fibroblastic disintegration in the inflamed areas was enhanced when the hormones were given in larger doses (10 to 50  $\gamma$ ).

When cortisone was given in the largest doses (150  $\gamma$ ) in the form of commercial Cortone (Merck), maximum fibroblastic disintegration was produced, and

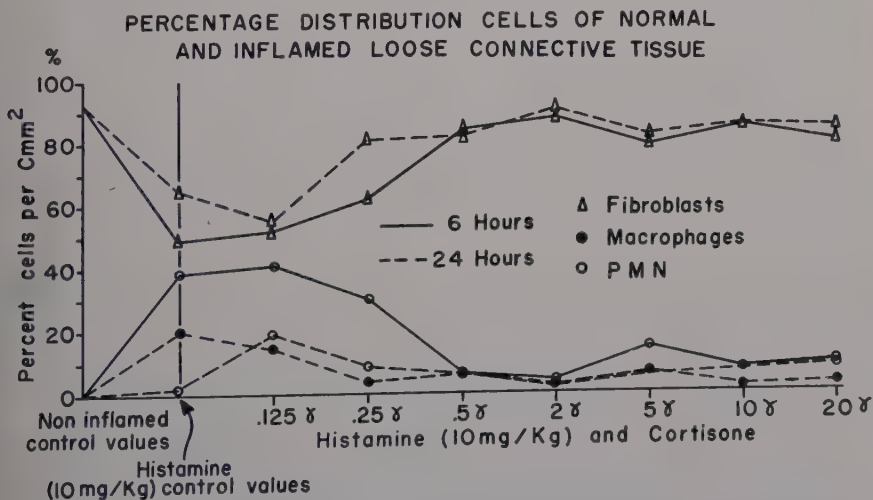


FIGURE 1. Percentage distribution of fibroblasts, macrophages, and PMN's in loose connective tissue of animals given topical doses of histamine and graded doses of cortisone. Hormone concentrations effective in reducing PMN infiltrations are also effective in protecting fibroblasts and minimizing phagocytic activity.

it was found that the cortisone suspending agent itself was phlogogenic when injected alone.<sup>6</sup> Large doses of the crystalline hormone (1 mg. or more) when implanted free of suspending agent in areolar tissue produced complete fibroblastic destruction at the site of implantation.<sup>1, 6</sup>

The polymorphonuclear leukocytes began to infiltrate the inflamed areas within two hours after histamine injection in control animals (FIGURE 1). There was a continuous increase in these cells up to approximately six hours (FIGURE 1). Later stages were characterized by macrophage response (FIGURE 1). When cortisone was given in graded doses along with the histamine dose to adrenalectomized animals, the entrance of PMN's and macrophages into the area of inflammation was diminished.

The decrease in number of PMN's over the lower range of doses appeared to be linear. Accordingly, graded amounts of cortisone were given to animals between 0.125  $\gamma$  and 0.5  $\gamma$ . The amount of histamine was held constant at 10 mg./kg. The linear regression curve for cortisone dose, plotted against the percentage of PMN's/mm.<sup>2</sup> tissue is given in FIGURE 2. Cortisone doses greater than 0.5  $\gamma$  did not result in any further inhibition of inflammation.

### *Pyrogen Induced Inflammation*

Three groups totaling 170 adrenalectomized male CBA mice were used in these experiments. Mice of group 1 were given subcutaneously 0.25  $\gamma$  of a pyrogen mixed with graded doses of cortisone. Pyrogen (Pyromen, Pseudomonas, Baxter) alone was given to adrenalectomized animals which served as controls. A subcutaneous injection of 0.125  $\gamma$  of pyrogen, mixed with 0.5 and 5  $\gamma$  of Cortone, respectively, was given to animals of group 2. The loose connective tissue spread method as outlined above was used for both groups 1 and 2.

A subcutaneous injection of 0.125  $\gamma$  of Pyromen was employed in group 3. Immediately after pyrogen administration, cortisone in graded doses (0.0625, 0.25, 0.5, 1, 5, 10, 20, 50, 100, and 200  $\gamma$  and 1 mg.) was given by tail vein injection. Control animals received either pyrogen (subcutaneous) alone, or with intravenously injected saline. Loose connective tissue spreads were prepared six hours later from the area of pyrogen administration. Only a total polymorphonuclear leukocyte count was performed in this group of animals.

The microscopic examination of control spreads taken six hours after injection of 0.25  $\gamma$  of pyrogen revealed not only an extensive cytolytic action of this drug on fibroblasts, but also the presence of large numbers of polymorphonuclear leukocytes in the areolar tissue (FIGURES 3 and 10). It should be emphasized that, in spreads of untreated control animals, which served as normal controls for all our experiments, no neutrophilic granulocytes were found. A decrease in number of mast cells of all pyrogen-treated control animals, in addition to a rupturing and degranulation of the surviving tissue basophils, was also observed. This finding confirms the observation of others.<sup>7</sup>

Administration of cortisone alone with pyrogen to the experimental animals resulted not only in a marked inhibition of the destructive effect of the pyrogen upon fibroblasts (FIGURES 3 and 4), but also in a diminution of the number of invading polymorphonuclear leukocytes (FIGURES 3 and 4), which was depend-

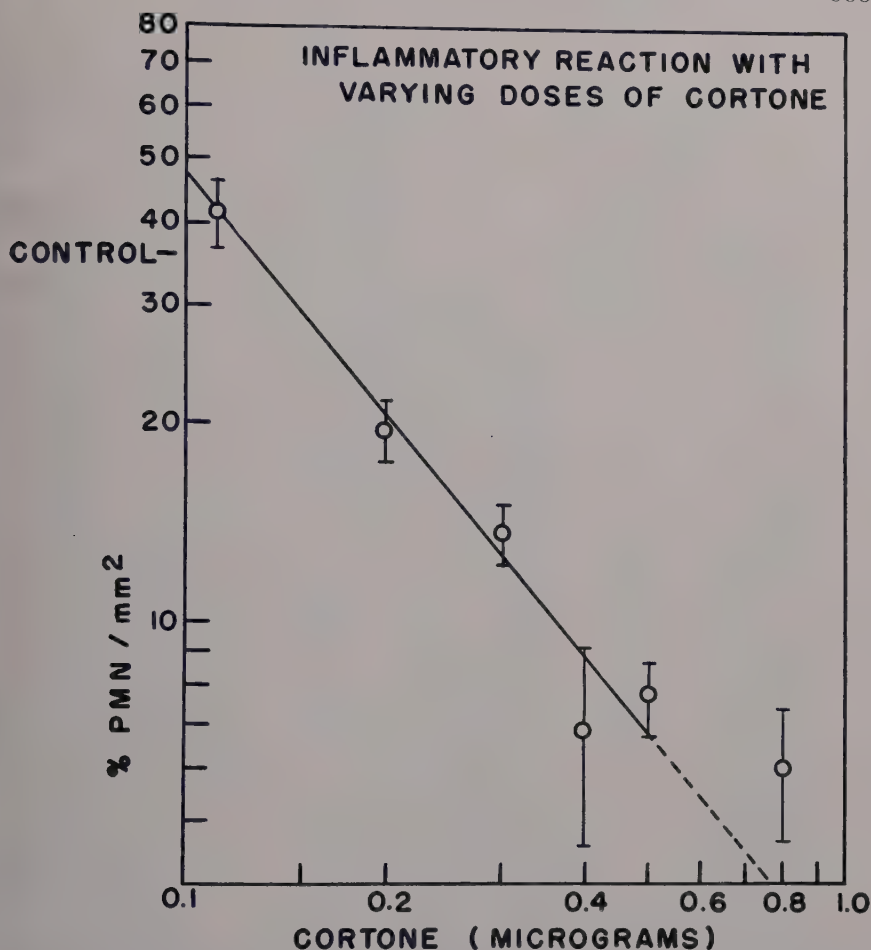


FIGURE 2. Log of the percentage of PMN's/mm.<sup>2</sup> in inflamed subcutaneous connective tissue of adrenalectomized mice six hours after topical administration of 0.2 mg. histamine diphosphate and graded doses of Cortone. Vertical bars indicate the S. E. of each dose on either side of the mean. Since this is a log-log dose response curve, the standard errors for the larger doses appear greater below than above each point. The curve was fitted by the method of least squares.

ent on the concentration of the hormone employed. The majority of mast cells survived treatment with the cortisone-pyrogen mixture. Smaller pyrogen doses as employed in group 2 mice were somewhat less effective in producing diapedesis of granulocytes and tissue damage. The amount of cortisone necessary to suppress inflammatory reactions was also small in this experiment.

Intravenous administration of cortisone to animals treated topically with pyrogen was as efficient in preventing leukocyte diapedesis and fibroblast destruction as was the subcutaneous administration. Pyrogen proved to be significantly more lytic for fibroblasts than the other phlogistic substances employed in these experiments (FIGURE 10).

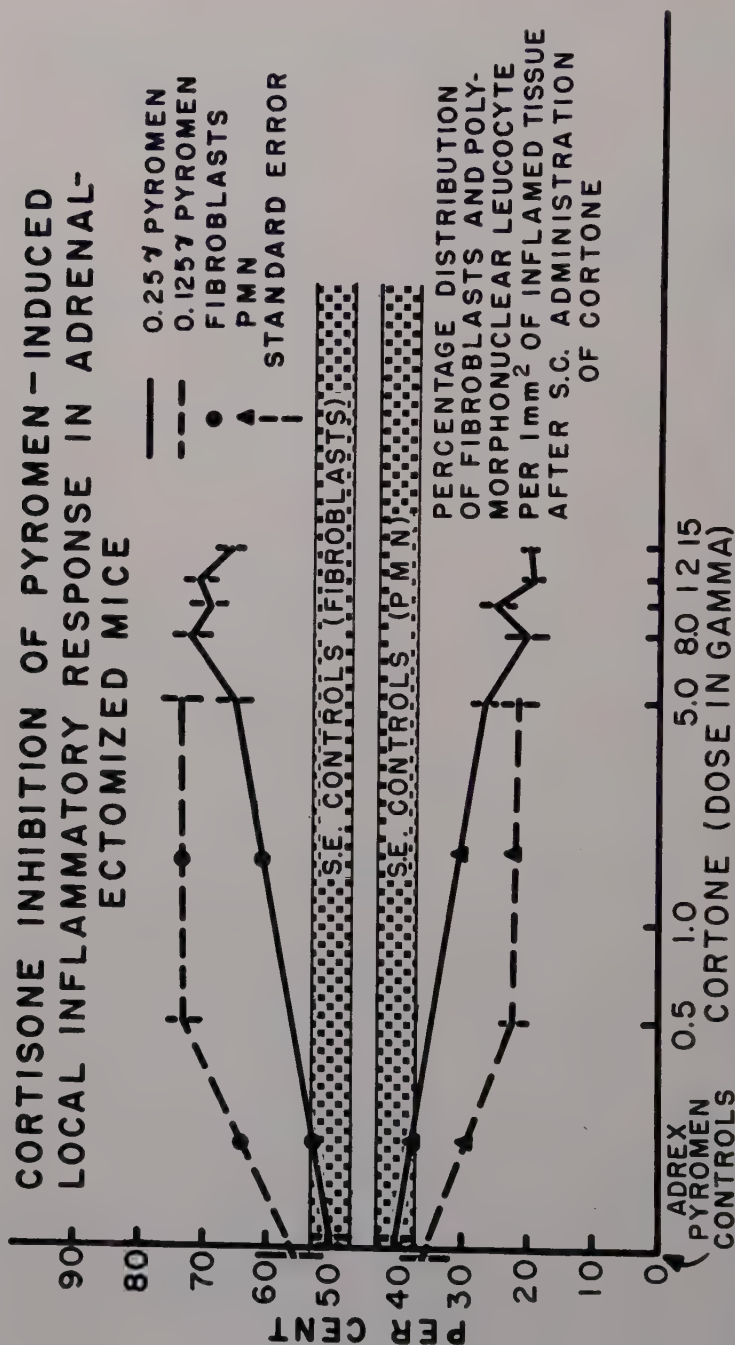


FIGURE 3. Percentage distribution/mm.<sup>2</sup> of fibroblasts and PMN's of loose connective tissue inflamed by topical administration of two different doses of pyrogen. Antiphlogistic activity of cortisone is shown by the decrease in number of PMN's/mm.<sup>2</sup> when increasing doses of hormone were used. The decrease of PMN's was greater when the smaller dose of pyrogen was used. Note that preservation of fibroblasts is also greater in animals given the smaller dose of inflaming agent.



# CORTISONE INHIBITION OF PYREMEN (0.125 γ S.C.)-INDUCED LOCAL INFLAMMATORY RESPONSE IN ADRENALECTOMIZED MICE

TOTAL NUMBER OF POLYMORPHONUCLEAR LEUCOCYTES PER mm<sup>2</sup> OF INFLAMED TISSUE AFTER I.V. ADMINISTRATION OF CORTONE

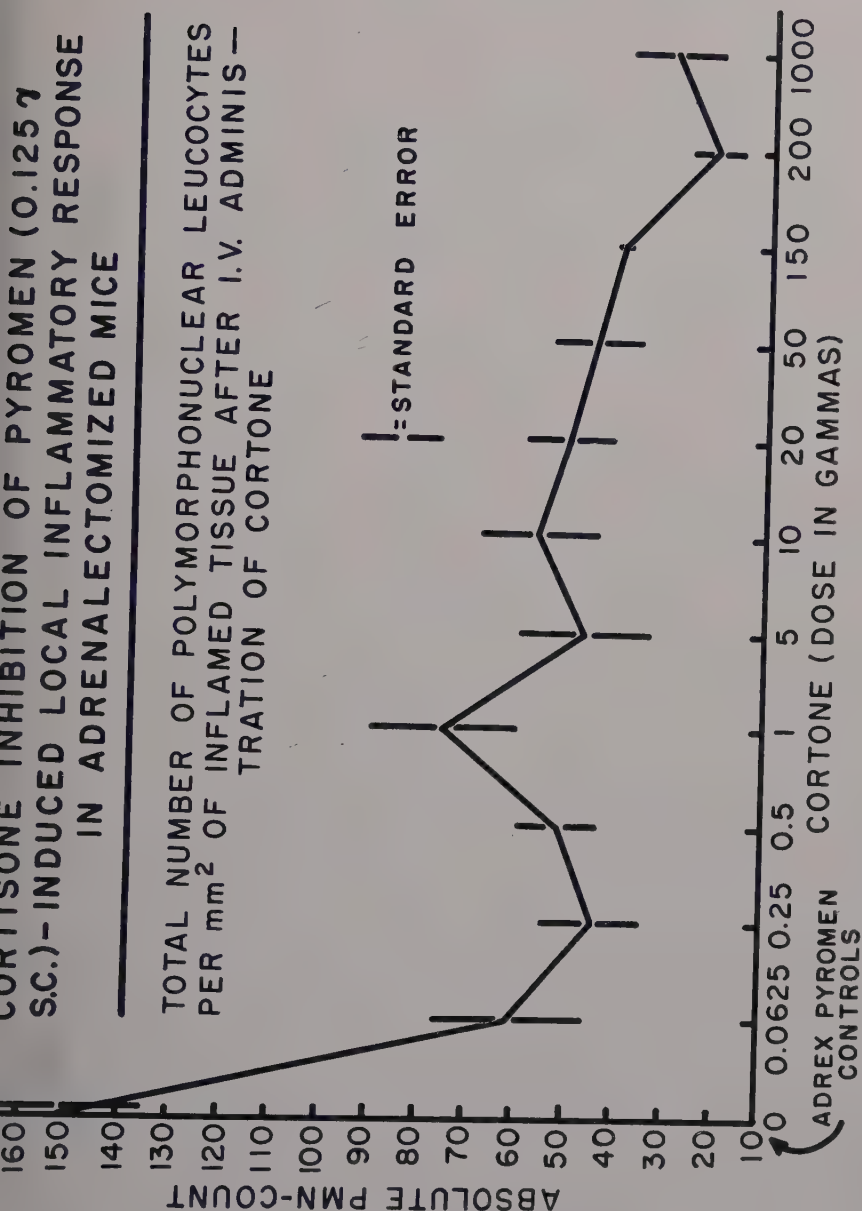


FIGURE 4. Dosage response of graded amounts of intravenously administered cortisone on total number of PMN's found in inflamed loose connective tissue after topical administration of 0.125 of pyrogen. It is evident that intravenous administrations of hormone is as effective in reducing the influx of PMN's as is topical administration, and the antiphlogistic activity is provided over a wide dosage range.

TABLE 1  
MEANS AND STANDARD ERRORS OF PERCENTAGE OF PMN'S/MM.<sup>2</sup> OF INFLAMED TISSUE TREATED WITH  
GRADED DOSES OF VARIOUS STEROID HORMONES

$\gamma$	Control	0.0001	0.0005	0.001	0.0015	0.002	0.003	0.005	0.01	0.05	0.1	1.0	10.0	N
F	34.9 $\pm 1.9$	34.3 $\pm 3.9$	27.2 $\pm 3.5$	25.8 $\pm 2.9$	24.7 $\pm 4.1$	22.7 $\pm 4.7$	18.0 $\pm 3.9$	17.6 $\pm 3.5$	13.6 $\pm 3.9$	12.9 $\pm 3.4$	15.8 $\pm 2.0$	12.8 $\pm 6.0$	10.1 $\pm 3.3$	95
$\gamma$	Controls	0.05		0.1	1.0		2.5	3.0	5.0		10.0	20.0		N
E	39.1 $\pm 3.4$	26.0 $\pm 3.0$		21.1 $\pm 1.9$	16.5 $\pm 1.5$						14.4 $\pm 2.9$	17.0 $\pm 3.6$		53
B	45.6 $\pm$			35.3 $\pm 4.1$	30.9 $\pm 5.7$		20.4 $\pm 3.1$		31.3 $\pm 11.8$		27.3 $\pm 3.9$	27.6 $\pm 1.2$		52
S	37.0 $\pm$			24.9 $\pm 4.0$	33.7 $\pm 5.0$		31.1 $\pm 3.0$	21.4 $\pm 2.2$	15.2 $\pm 2.1$		15.8 $\pm 2.4$	12.7 $\pm 4.3$		100
Not F	30.8 $\pm$			24.0 $\pm 3.2$	24.8 $\pm 1.4$				17.6 $\pm 4.1$		14.9 $\pm 1.9$	18.8 $\pm 1.9$		53
A	37.8 $\pm$			29.5 $\pm 4.7$	32.3 $\pm 3.5$				21.3 $\pm 7.0$		27.0 $\pm 6.6$	17.7 $\pm 5.4$		47
D	35.8 $\pm$			26.0 $\pm 2.9$	29.8 $\pm 3.0$						34.6 $\pm 3.5$			28
L	38.8 $\pm$			42.2 $\pm 3.2$	30.0 $\pm 7.7$						35.6 $\pm 4.7$			12
DAC	33.3 $\pm$	36.1 $\pm 6.3$		25.7 $\pm 1.9$	35.7 $\pm 3.8$						34.7 $\pm 5.1$			44
DCA	34.8 $\pm$			30.8 $\pm 1.8$	31.6 $\pm 4.1$						26.6 $\pm 3.4$			15

*A Comparison of the Antiphlogistic Potencies of Some Steroid Hormones*

For the study of the relation of structure of adrenocortical steroids to antiphlogistic activity, a total of 499 adrenalectomized CBA mice were injected with 0.25 cc. of 1 per cent gelatin suspended in pyrogen-free saline. The methods used in these studies were the same as those described above. The following steroid hormones were tested in these experiments: compound A acetate (11-dehydrocorticosterone acetate); compound B (corticosterone); compound E acetate (11-dehydro-17-hydroxy-corticosterone acetate); compound F acetate (17-hydroxycorticosterone acetate); compound S acetate (17 alpha hydroxy desoxycorticosterone, 21 acetate); compound L (allo pregnan 3 beta, 17 alpha diol 21); DCA (11-desoxycorticosterone acetate); compound D, (allo pregnan 3 beta, 17 alpha, 21 triol, 11, 20 dione); and Nor F (19 nor 17 alpha hydroxy corticosterone).

In addition to the compounds listed above, which were assayed for their anti-inflammatory activity, several hormones (DCA, compounds D and L) were tested for a possible anticortisone activity.

As indicated above, 0.25 cc. of a 1 per cent solution of gelatin was used as a standard inflaming stimulus. Gelatin, in previous experiments, was found to be a mild inflaming stimulus. The data (TABLES 1 and 2) show that the gelatin dose employed produced 34.9 per cent PMN's mm.<sup>2</sup> in non-hormone-treated adrenalectomized control animals six hours after injection.

In order to insure that some weak antiphlogistic activity would not be missed, preliminary experiments were performed by giving a range of hormone doses at multiples of 0.01  $\gamma$ . This preliminary experiment gave us information concerning the optimal dosage range over which we might expect a linear regression curve. Five to eight animals were given each of the hormone doses shown in TABLE 1 and in FIGURES 5 and 6. Fifteen non-hormone-treated adrenalectomized animals were given the standard inflaming dose of gelatin. This number of animals was used for control values for each of the separate assays (TABLE 1). The ranges of doses representing the portion of the curve used for dose-response phenomena are indicated in TABLE 1 and also in FIGURES 5 and 6. The results of each of the assays were evaluated according to the method of least squares. The ranges of doses, numbers of animals, statistical significance of the parallelism of the regression lines, and slope values are given in TABLES 1 and 2, and in FIGURES 5 and 6.

In all instances, the basis of comparison for the antiphlogistic potency of hormones tested was the anti-inflammatory activity of hydrocortisone (compound F). Therefore, in order to determine the effect of experience or inexperience of the operator on the results of counting the slides, an analysis of variance for this method of measurement was performed. Each of three people, J, K, and G, performed counts of PMN's on the same spreads on slides taken from animals which had received the standard inflaming stimulus (1 per cent gelatin) and seven graded doses of hydrocortisone. The same individuals also made differential counts on spreads taken from animals given nor-hydrocortisone. Consequently, each of these three individuals counted the same areas for two different compounds at each of the different doses. In all in-

## ANTIPHLOGISTIC POTENCY OF STEROIDS

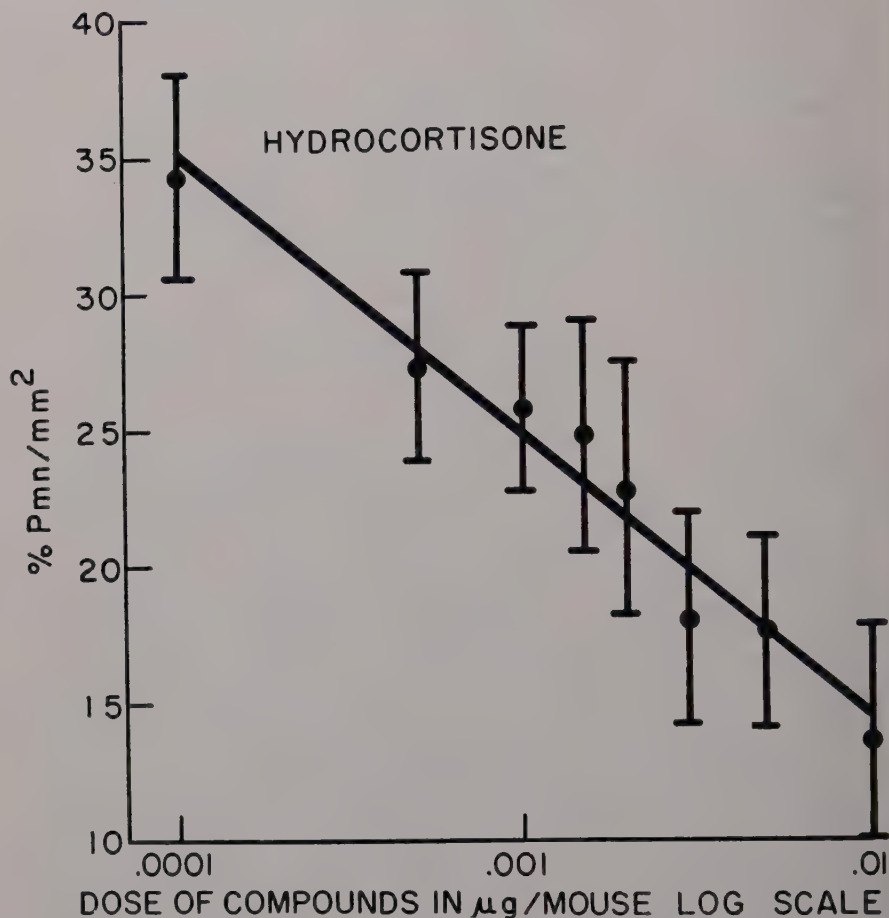


FIGURE 5. Linear regression curve of percentage of PMN's in inflamed areolar tissue six hours after injection of .25 cc of 1 per cent gelatin in saline and graded doses of hydrocortisone. Standard errors are indicated by the vertical bars.

stances, the slides were given code numbers, and none of the people performing the counts knew the results obtained by others. An analysis of the variance in the resulting percentage of polymorphonuclear counts gave the following mean sums of squares. Among the people, the sum of the squares was equal to 72.6 with 2 degrees of freedom. Between treatment levels it was equal to 364.7 with 6 degrees of freedom. Within doses, *i.e.*, the error, the sum of squares, was equal to 41, with 54 degrees of freedom. The ratio of error among people to total error is equal to 1.77 with 2 and 54 degrees of freedom. The *P* value was  $>.05$ . Therefore, there is not a significantly greater amount of variation in the measurements made by the different people than in one person's own measurements of the slides from one level of treatment.



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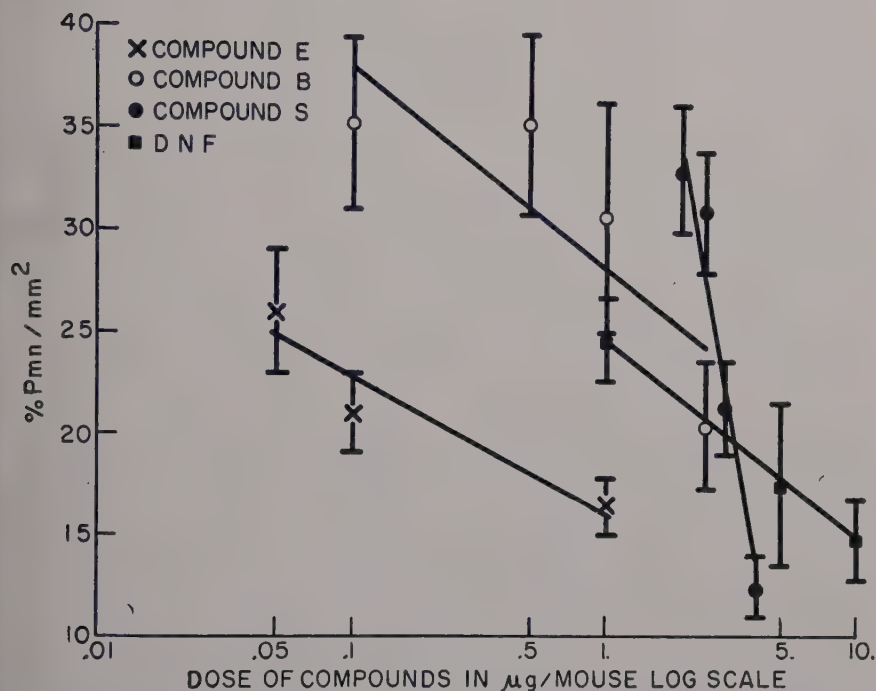


FIGURE 6. Percentage of PMN's/mm.<sup>2</sup> in areolar tissue inflamed with .25 cc. of 1 per cent gelatin in saline and simultaneously treated topically with graded doses of either compound E, B, S or Nor F. Vertical bar indicate standard errors. It will be observed that the slopes of compounds E, B, and Nor F are parallel.

The linear regression curve for eight different doses of hydrocortisone is shown in FIGURE 5. It may be seen that the curve is linear over the dosage range from .001  $\gamma$  to .01  $\gamma$ . It was shown in 1952 (8) that from .01  $\gamma$  to approximately 1  $\gamma$  the curve does not regress further but stays flat. Above 10  $\gamma$ , the inflammatory response may actually return, so that instead of inhibiting inflammation at this point, it may be enhanced, or it may not differ significantly from the control values.<sup>8</sup> In FIGURE 6, the regression lines for cortisone, corticosterone, substance S and nor-hydrocortisone are shown. The dosage range is from .05  $\gamma$  to 10  $\gamma$ . The regression lines for cortisone, corticosterone, and nor-hydrocortisone are parallel to that found for hydrocortisone. Consequently, a comparison of the potency of these hormones could be made. The linear regression line for substance S is not parallel to those of the other compounds, which demonstrated antiphlogistic potency. According to the usual interpretation, it is assumed that the mechanism of action of different compounds is similar if the regression lines are parallel. According to this point of view, the mechanism of antiphlogistic action of hydrocortisone, cortisone, corticosterone, and nor-hydrocortisone is essentially the same. Substance S, however, which has a very steep regression line and demonstrates no antiphlogistic potency until a large amount of the hormone is available at the site, must

TABLE 2

SLOPE VALUES AND STATISTICAL DATA FOR LOG DOSE RESPONSE LINES OF PER CENT PMN'S/MM.<sup>2</sup> OF INFLAMED TISSUE TREATED WITH GRADED DOSAGE STEROID HORMONES

$$\frac{\sum y(x-\bar{x})}{\sum (x-\bar{x})^2} \sqrt{\frac{S.D.(L)}{(x-\bar{x})^2} \frac{\sum (y-\bar{y})^2}{\sqrt{N-2}} \frac{S.D.(L)}{b}} \quad \bar{y}-b\bar{x}$$

Compound	<i>b</i>	S.E. ( <i>b</i> )	S.D. (L)	$\lambda$	<i>a</i>	P <sub>b=0</sub> *	P <sub>b=bf†</sub>	<i>R</i> <sub>f/z</sub>	95% Fiducial limits
F	-10.41	2.5	10.1	.97	35.21	<<.01			
E	-6.51	2.2	6.3	.97	42.25	<<.01	> .1	78	26 to 233
B	-9.83	4.3	11.1	1.13	67.56	.03	> .1	2570	656 to 10,050
Nor F	-9.63	3.1	6.9	.72	63.06	< .01	> .1	933	133 to 3,160
S	-73.58	11.1	7.7	.10	57.07	<<.01	<<.01		

\* Probability (from student's *t* table) that *b* = 0 using *t* = *b*/S.E. (*b*).

† Probability (from student's *t* table) that the slope of the compound is the same as the slope of F using  $t = b - b_f / \sqrt{[S.E. (b)]^2 + [S.E. (b_f)]^2}$ .

be acting by some other mechanism than the other antiphlogistic hormones. The dosage ranges, their mean values, and standard errors for the percentage of PMN's/mm.<sup>2</sup> of inflamed tissue, as well as the total number of animals for each of these experiments are given in TABLE 1. In certain cases, fewer animals were used because small amounts of hormones were available.

The values for the slopes and the statistical validity for the log-dose response line of the per cent PMN's/mm.<sup>2</sup> of inflamed tissue are given in Table 2. Here, the slope values (*b*-TABLE 2) are given for the effective antiphlogistic hormones. The linear regression curve will decrease according to the value given for *b*. For instance, for compound F, the value for *b* is 10.41 (TABLE 2), which indicates that, for any unit increase in the log of the amount of compound F, the unit per cent PMN's will decrease 10.41.

It may be seen that the slopes for E, B, and Nor F are not significantly different from that of F, which is used as the line of reference (FIGURE 5, and TABLE 2). The slope of substance S, however, is significantly greater than the slope of F as may be seen in FIGURE 6 and TABLE 2 (*P* >> .01). The  $\lambda$  values (indices of precision) are consistent within the groups of assays, with the exception of substance S. The smaller  $\lambda$  for the line of substance S seems to be a direct result of the larger slope of this line. The standard errors for the slopes are all small with the exception of that found for corticosterone (TABLE 2). The probability that the slope for each compound is zero may also be observed from TABLE 2. Student's *t*-test shows that all of the slopes are significantly different from zero except that of B, which has a high standard error. The potency ratios of the various compounds as compared to hydrocortisone are also given in TABLE 2 with their 95 per cent fiducial limits.

*Structure Activity Relationships.* The structure activity relationships and the potency ratios of the effective antiphlogistic hormones tested are shown in FIGURE 7. In the right-hand column of this figure, the ineffective analogues of the potent antiphlogistic hormones are also given. It may be seen that the most effective of these hormones is hydrocortisone. Cortisone, according to this method of assay, is about 76 times less potent than hydrocortisone.

95% Fiducial Limits		$\lambda$ of Assay		Dose Range		Potency Ratio	Dose Range		Other Observations
<i>Cpd. F</i>					.0001 $\times$ TO 10 $\times$				
<i>Cpd. E</i>					.05 $\times$ TO 20 $\times$	F/E 76.8			
<i>Cpd. B</i>					1 $\times$ TO 20 $\times$	F/B 2440.0			
<i>Cpd. S</i>					.1 $\times$ TO 20				
<i>NOR. F</i>					.1 $\times$ TO 20 $\times$	F/NOR. F 933			
<i>Cpd. A</i>			.97					.1 $\times$ TO 20 $\times$	
<i>Cpd. D</i>			.97					.1 $\times$ TO 10 $\times$	LYMPHOCYTIC INVASION IN AREA OF INJECTION
<i>Cpd. L</i>			1.13					.1 $\times$ TO 10 $\times$	LYMPHOCYTIC INVASION IN AREA OF INJECTION
<i>11 Dihydro- alloclortisone</i>			.10					.1 $\times$ TO 10 $\times$	
<i>DCA</i>			.72					.1 $\times$ TO 20 $\times$	ANTICORTISONE EFFECT

FIGURE 7. Structure activity relationship of some antiphlogistic steroid hormones.

A comparison of the effective and ineffective hormones indicates that the greatest antiphlogistic activity was demonstrated by hydrocortisone. An analogue of hydrocortisone, 11-dihydroallocortisone, tested over approximately the same dosage range, was ineffective. It would seem, then, from an analysis of the structure of the effective and ineffective compounds, that the greatest antiphlogistic activity is manifest when a hydroxyl group is present at both 11 and 17, and when ring A is unsaturated. It was also observed that analogues of cortisone and hydrocortisone in which the A ring is saturated are completely without antiphlogistic effect. Corticosterone, which has a hydroxyl group at position 11, but does not have a hydroxyl group at 17, had a slight antiphlogistic effect. This compound was tested over the dose range of  $1\gamma$  to  $20\gamma$ . It will be noted that the 95 per cent fiducial limit is extremely large. Therefore, this compound is extremely variable in its antiphlogistic influence. The same findings apply to compound A, which was tested over an even greater dosage range. Although some antiphlogistic influence was observed among some of the treated animals, no linear regression line could be obtained for this hormone.

It is suggested, then, that the absence of an hydroxyl group at the 17 position markedly reduces the *consistency* of antiphlogistic effectiveness. On the other hand, the absence of a hydroxyl or oxy-group at 11 did not eliminate completely the anti-inflammatory effect, as is demonstrated for substance S. The absence of the hydroxyl group and reduction of ring A not only was devoid of anti-inflammatory effect, but induced a lymphocyte infiltration of the tissue.

In addition to the quantitative method used here, a simple screening procedure for anti-inflammatory activity may be used. Crystals of the antiphlogistic substances do not produce inflammation when implanted subcutaneously. Those which lack this influence may be markedly inflammatory. Compounds D, L, and 17 hydroxyl progesterone were all exceedingly phlogogenic. It is suggested then, that since 17 hydroxy progesterone does not have antiphlogistic influence, the presence of the hydroxyl group is essential for this activity. The presence of alpha and beta unsaturated ketone in ring A is essential when the 17 OH and the 21 OH are present. If 21 OH is lacking the compound is an ineffective antiphlogistic substance. Even if C-21 OH and C-17 OH are present and ring A is reduced in any position, however, the anti-inflammatory activity is absent.

*Anticortisone Effect.* The fact that desoxycorticosterone could inhibit some of the effects of the C-11 oxysteroids was noted in 1949.<sup>2</sup> It was shown in this laboratory that administration of DCA either prior to or concomitantly with cortisone inhibited the anti-anaphylactic influence of cortisone.<sup>2</sup> Thus, it became clear that this anticortisone activity was not mediated by way of pituitary suppression. Later<sup>3, 3</sup> it was also demonstrated that DCA exerted an anticortisone effect in inflammation. The authors have examined compounds D and L for possible anticortisone activity and found that these compounds were unable to inhibit the anti-inflammatory effect of cortisone.

In other experiments in which DCA and cortisone were given mol for mol over a graded series of dosages to animals in which the inflaming stimulus was held constant, it was found that DCA provided a mol for mol inhibition over a



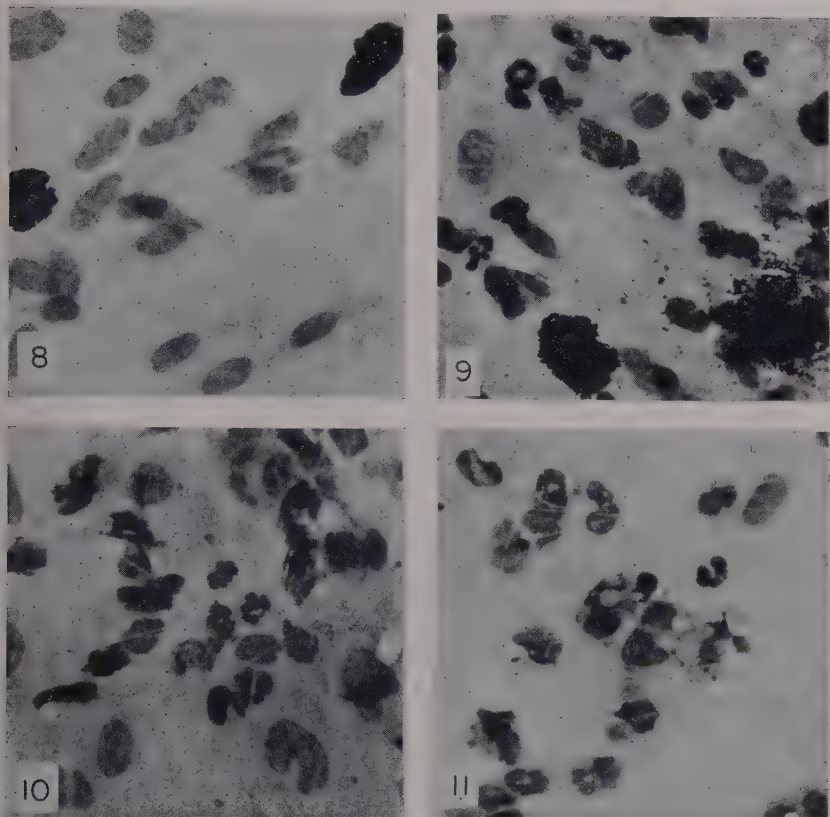


FIGURE 8. Loose connective tissue spread of untreated 16 weeks old CBA male mouse. Stained with May Grünwald-Giemsa. Magnification  $\times 600$ .

FIGURE 9. Areolar tissue of mouse given 0.2 mg. histamine in .85 per cent sodium chloride subcutaneously six hours after treatment. Note influx of nonautochthonous cells, slight shredding of fibroblasts, and degeneration of mast cell on lower right hand corner of picture. Staining and magnification as in FIGURE 8.

FIGURE 10. Areolar tissue of mouse treated with 0.125  $\gamma$  of pyrogen subcutaneously six hours after treatment. Note extensive cytolytic action of drug on fibroblasts. Granules in ground substance probably come from disintegrating mast cells. Staining and magnification as in FIGURE 8.

FIGURE 11. Areolar tissue of mouse treated with 0.25 cc. of 1 per cent gelatin suspended in saline. Macrophage response 12 hours after treatment. Staining and magnification as in FIGURE 8.

considerable dosage range (unpublished observations). This suggests that DCA inhibits cortisone in a competitively inhibitory manner. Recently Selye<sup>3</sup> has found that aldosterone also exerts an "anticortisol" effect which is approximately equivalent to that of DCA. He concludes that this finding supports his "corticoid-antagonism" theory.

In the authors' opinion, the direct inhibitory influence of DCA or of aldosterone on the anti-inflammatory effects of cortisone and hydrocortisone may not provide the only explanation of the capacity of the mineralocorticoids to provide enhanced degrees of inflammation. It appears that DCA may also act by increasing the susceptibility of the cells to the inflaming stimulus with consequent hyper release of those phlogogenic substances which trigger the inflammatory response (unpublished observations).

*Mechanism of Hormonal Anti-Inflammatory Effect*

If inflammation is considered as a single entity, it is impossible to ascertain which of the concatenation of events composing this response is moderated by adrenocortical hormones. Cellular injury is not in itself an inflammatory response. It is rather a stimulus to the development of the series of events grouped inclusively under the term inflammation. For example, burned tissue is not an inflammation, but the reaction to the burned tissue is an inflammation. It is clear, then, that adrenocortical hormones do not inhibit the production of the initiating stimulus which triggers the chain reaction of inflammatory response.<sup>8, 2, 9</sup> These hormones probably exert their antiphlogistic action by interrupting the chain reaction of cellular destruction triggered by the injurious stimulus. According to this point of view, inhibition of inflammation is exerted by preventing the release and enhancing the removal of substances coming from injured cells.<sup>2, 10</sup>

It is apparent from an analysis of the literature,<sup>2</sup> as well as from work performed in our laboratory, that cortisone does not completely prevent the release of products of cellular injury.<sup>2</sup> For example, we have demonstrated that cortisone treatment does not prevent the release of anaphylotoxin in anaphylactic shock or in local anaphylaxis.<sup>11</sup> It also does not prevent the Schultz-Dale reaction.<sup>11</sup> Cortisone administration, for example, inhibits histamine flare but not the release of histamine which takes place following the administration of a histamine releasing drug (reviewed by Dougherty<sup>2</sup>).

We have suggested that the antiphlogistic adrenocortical hormones exert their effects by enhancing the resistance of surviving noninjured cells<sup>2, 10</sup> to the cytotoxic action of phlogogenic agents. If the antiphlogistic effect is exerted in this manner, the initially released cytotoxic substance that potentiates the inflammatory response and continues the chain reaction of cellular destruction would in turn be decreased. Recently, Eyring and Dougherty<sup>10</sup> proposed that, in inflammation, "There is always a race between autocatalytic cell destruction in which the breakdown of one cell promotes the breakdown of another, pitted against the inactivating of the destructive products liberated by the broken cell." These authors<sup>10</sup> propose a unified hypothesis suggesting that it is not necessary to assume that different chemical substances perform specific functions which induce each of the phenomena of the inflammatory response. They suggest that all of the events in acute inflammation can be explained by cellular injury resulting from a lack of stabilization of the cell membrane. According to this hypothesis, the antiphlogistic hormones act by preventing cellular destruction by stabilizing the impermeable state of the cell membrane.

In line with these observations, it has been shown that hydrocortisone tends to produce a rounding-up of the fibroblasts in hormone treated noninflamed tissue (FIGURE 13). Fibroblasts which survive in an area of inflammation in intact or in hydrocortisone-treated animals are usually of this rounded-up type.

Other experiments utilizing cytochemical and radioautographic methods show that fibroblasts concentrate the hydrocortisone within their cytoplasm. The histochemical technique used is supposed to be specific for demonstration

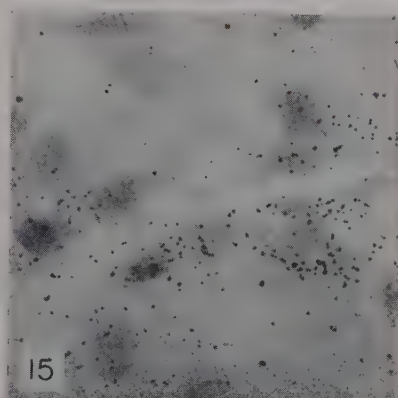
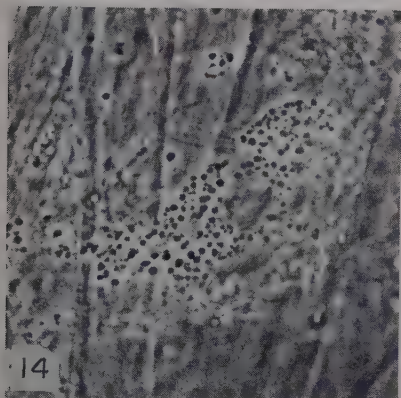
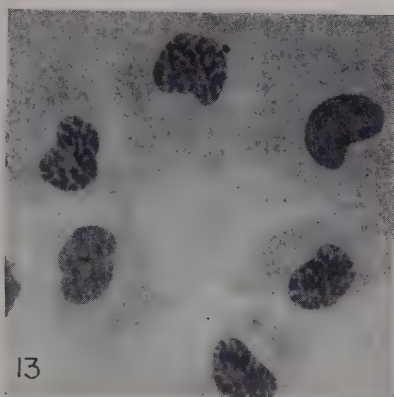
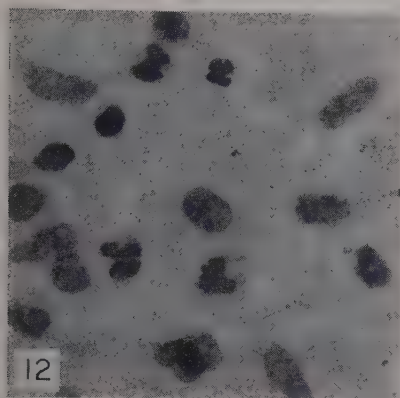


FIGURE 12. Loose connective tissue six hours after 0.125  $\gamma$  of pyrogen and 1  $\gamma$  cortisone acetate. Note preservation of fibroblasts and reduction in number of PMN's. Staining and magnification as in FIGURE 8.

FIGURE 13. Loose connective tissue of mouse given 250  $\gamma$  of compound F in sesame oil intraperitoneally for four days. Note rounding up of fibroblasts. Some cells are in early prophase. Note also characteristic granularity of the ground substance. Staining May Grünwald-Giemsa. Magnification,  $\times 1200$ .

FIGURE 14. Intracellular inclusion in fibroblast of loose connective tissue of mouse given 25  $\gamma$  topically of compound F in saline. Seligman Reaction. Six hours after treatment. Dark phase microscope observation. Magnification  $\times 1330$ .

FIGURE 15. Detail radioautogram of areolar tissue from mouse given 10  $\gamma$  C-14 labelled hydrocortisone topically. Note the dark grains concentrated over the cytoplasm which correspond to C-14 concentrations. Areolar tissue and superimposed strip film (Eastman Kodak) exposed for eight months and stained with hematoxylin and eosin. Magnification  $\times 600$ .

of ketosteroids.<sup>12</sup> Fibroblasts of animals given 25  $\gamma$  of hydrocortisone topically showed marked cytoplasmic granulation six hours after hormone treatment when observed through the phase microscope. The technique used for radioautography is a modification of a method described by Arnold and Jee.<sup>13</sup> Radioactive hydrocortisone (C-14 labeled) was used in these experiments. Ten  $\gamma$  of the substance was given topically as described above. Six hours after administration, spreads were made and exposed with photographic emulsion for eight months. The radioautogram was stained with hematoxylin and eosin. The tracks shown in FIGURE 15 were concentrated in cytoplasm of fibroblasts. Few extracellular tracks, apart from the fibroblasts, were found in ground substance. Due to the extensive washings of the tissue preparations, the cyto-



plasmic staining in these radioautograms are relatively weak and somewhat hard to visualize.

In summary, then, it is suggested that hydrocortisone administered topically tends to concentrate after a few hours within cytoplasm of the fibroblasts. At this stage, the cell tends to round up, increasing its cytoplasmic basophilia, and becomes more epitheloid in appearance. These cells also appear to be more resistant to fibroblastolysis.

### *Acknowledgments*

We wish to thank Doctors Augustus Gibson and Elmer Alpert of Merck and Company for the generous supply of most of the hormones used in this and other of our investigations. We also wish to thank the laboratories of Syntex in Mexico, D. F., Mexico, for furnishing us with compounds L, D, 11-dihydro-allocortisone and Nor F. We are grateful to Doctor William F. Windle, formerly of Baxter Laboratories, for supplying us with the pyrogen used in these studies.

We are grateful to Katherine Seymour for her most able technical assistance and to Mrs. Elizabeth Chamberlin for her consultation in designing and evaluating statistically the results of these investigations. We are also grateful to Webster Jee for his help in the radioautographic technique used.

### *References*

1. DOUGHERTY, T. F. & G. L. SCHNEEBELI. 1950. Role of cortisone in the regulation of inflammation. *Proc. Soc. Exptl. Biol. Med.* **75**: 854.
2. DOUGHERTY, T. F. 1954. The mechanism of action of adrenocortical hormones in allergies. *Progr. Allergy* **4**: 319.
3. SELYE, H. & G. HEUSER. 1954. Fourth annual report on stress. *Acta, Inc.* Montreal, Canada.
4. KOLOUCH, F., JR. 1939. The lymphocyte in acute inflammation. *Am. J. Path.* **15**: 413.
5. DOUGHERTY, T. F. 1951. The protective role of adrenocortical secretion in the hypersensitive state. *Pituitary-Adrenal Function. Am. Assoc. Advancement Sci.* : 79.
6. SCHNEEBELI, G. L., T. F. DOUGHERTY & S. LOEWE. 1951. Production of cytoplasmic azurophilic inclusions in connective tissue cells by suspending agents. *Proc. Soc. Exptl. Biol. Med.* **77**: 407.
7. STUART, E. G. 1952. Mast cell responses to anaphylaxis. *Anat. Record* **112**: 92.
8. DOUGHERTY, T. F. 1952. Studies of the antiphlogistic and antibody suppressing functions of the pituitary adrenocortical secretions. *Recent Progress in Hor. Research* **7**: 307. Academic Press, New York, N. Y.
9. DOUGHERTY, T. F. 1953. The effect of cessation of treatment with large doses of antiphlogistic adrenocortical hormones on circulating antibody and parenchymatous allergic lesions. *Rev. can. biol.* **12**: 305.
10. EYRING, H. & T. F. DOUGHERTY. Molecular mechanisms in inflammation on stress. *Am. Scientists*. In press.
11. DOUGHERTY, T. F. 1950. The relation of adrenal cortical hormones to the hypersensitive state. *In Adrenal Cortex* : 85. *Trans. 2nd Conf. Josiah Macy, Jr. Found.* New York, N. Y.
12. SELIGMAN, A. M. & R. ASHBEL. 1952. Histochemical demonstration of ketosteroids in adrenal cortical tumors with or without an associated cushing syndrome. *Endocrinology* **50**: 338.
13. ARNOLD, K. S. & W. S. S. JEE. 1954. Embedding and sectioning undecalcified bone and its application to radioautography. *Stain Technol.* **29**: 225.



*Discussion of the Paper*

DOCTOR GREGORY PINCUS (*Worcester Foundation for Experimental Biology, Shrewsbury, Mass.*): I should like to ask Doctor Dougherty if ACTH gives a result similar to that of the adrenal steroids, and I should also like to know if he has had any experience with aldosterone because, very obviously, its greater potency, if the function is related to electrolyte activity, should make it very valuable.

The deduction about the mechanism requires one to give consideration to compound F. That is the most interesting compound of all, because this extraordinary steep slope which differentiates it from all the others makes me wonder if one might not find peculiarly interesting reactions in other types to be studied.

DOCTOR DOUGHERTY: We have never given an ACTH locally, that is, to find its expressive effect. Of course, ACTH administered to a nonadrenalectomized animal will have an effect. This we have not assayed. It is to be realized that this is all done in adrenalectomized animals, so there is a removal of the endogenous hormone supply.

We have not tried any aldosterone. I intend to get some but have been unable to do so thus far.

I think the point that compound S has little if any effect on inflammation is very interesting. We tested it twice because we were quite surprised at the result. Compound S has no effect on lymphatic tissue at all in relatively huge doses and, after seeing this, we went back over everything with compound S and could get no effect on thymic weight; no effect on lymph nodes; no effect on the lymphocyte count with compound S.

The only possible features that might be of interest from other people's work and that of Doctor John Plager, working with Doctor Leo Samuels of Utah, have suggested that there might be a conversion in the peripheral tissue as hydrocortisone, and the possibility that one would have to give a certain level of dose in order that even a minute quantity might become converted to F, so that this might explain the tremendous amount one would have to give, and suddenly, when one gets this effect, this tremendous drop occurs, so the line may have a steeper slope simply because it is necessary to build up a certain quantity in order to get a sufficient amount converted.

If Doctor Plager and others are right in that the tissue might convert S to F, this might be an explanation.

QUESTION: Many years ago, Menken showed in his studies on inflammation that, on injecting adrenal cortical extract and, more recently, cortisone, there was an interference with the lymphatic supply. He injected trypan blue into the ear vein of a rabbit and injected compound F into the area of the shaved skin of the animal with the inflammatory stimulus injected at the same time.

We have repeated this experiment and have been able to demonstrate that the injection of cortisone into the skin is followed by an apparent inhibition of the access of trypan blue to that area.

Do you think some of your findings could be explained on the basis of the

fact you have these constricting effects, or do you think it might affect the leukotaxine to which Doctor Menken has paid so much attention?

DOCTOR DOUGHERTY: Of course I would say we don't know. My feeling is that it affects the action of leukotaxine or of a substance release which figures in inflammation. I like that point of view better than the view that this effect is lymphatic, but I would say that we really don't know.

QUESTION: Have you made studies with trypan blue?

DOCTOR DOUGHERTY: We have done the same thing that Doctor Benjamin has described, but with little result.

QUESTION: Not being a statistician, but one who has dealt with statistics on occasion, I must point out that a value of the lambda value of .97 would mean that, for assay purposes, we want to decide whether one compound is twice as active as another. This means using hundreds of animals, if one can do it at all and, actually, in your comparisons of relative activities you have, in some instances, differences from 6-fold to 100-fold. Is this an assay procedure at all?

DOCTOR DOUGHERTY: Of course the value of lambda has real value in comparing one assay to another, but essentially it is just a question of actually being a meaningless term. It doesn't say anything. It is just a value, so if you run three assays and have a lambda value of 4.01 and .2 on the other, you suspect the 4 figure. The actual value of it is highly questionable. Some fairly good mathematicians can't understand why biometricians even use it.

It seems to me to be of value only in telling one that if one does three assays, and one value of lambda shows which is quite outside of the range of the other two, there is something wrong either with the other two or with this one. But as to whether it is a good assay method or not, it really doesn't tell very much, because the slope may actually just be that way. For instance, most of the growth hormone assays run in the neighborhood of a very high lambda, 2, 3, and 4, and yet they are all we have, and it is impossible to say this is 100 per cent accurate. I don't know whether it is good assay or not, but it has worked so far, and I like it.

COMMENT: I should like to say we have used this method of Doctor Dougherty's for the last four years, and can confirm his results, for the most part. We should possibly vary somewhat in the potency difference between the various steroids but, as far as the basic method goes, we can confirm that this is a good experimental tool.

COMMENT: I should like to comment on Doctor Pincus' question about ACTH. Inflammatory exudates, as is well known, start at the initial stage of an outline pH, and if you take an alkaline agent and mix it with hydrocortisone or cortisone, the capillary action is depressed. When one takes an acid exudate mixed with cortisone or hydrocortisone, there is no such depression and, when we mix it with ACTH, we determine this other factor. Now this exudate is oppressed by ACTH and it seems to be a direct effect. That has been tried on adrenalectomized rats and we have found the same oppression by ACTH. I am a little hesitant about one thing, and that is mainly the purity of the ACTH. We are, of course, resorting to commercial ACTH and I think these results will have greater value when we have pure ACTH.

## Part II. Use of Cortisone, Hydrocortisone, and Certain Synthetic Steroids in Systemic Disease

### PRESENT STATUS OF HYDROCORTISONE AS A THERAPEUTIC AGENT IN RHEUMATOID ARTHRITIS

By Edward W. Boland

*Los Angeles, Calif.*

When hydrocortisone was made available to us for clinical trial in the spring of 1951, three relevant facts seemed established: (1) hydrocortisone was the principal, probably the natural, glycogenic steroid secreted by the adrenal cortex;<sup>1-9</sup> (2) the hormone was considerably more potent than cortisone in certain metabolic activities, as measured by laboratory experiments in animals;<sup>10, 11</sup> and (3) it possessed antirheumatic activity, this property having been demonstrated in 1949 by Hench, Kendall, Slocumb, and Polley in a single rheumatoid arthritic patient given 900 mg. of the substance over a 12-day period.<sup>12</sup>

These considerations, together with the observation that long-term cortisone therapy failed to provide satisfactory improvement in a large proportion of patients, stimulated our interest in investigating the possible merits of hydrocortisone therapy in rheumatoid arthritis. The chief obstacle to better results with cortisone was the frequent intervention of troublesome side reactions, especially in patients suffering from severe forms of the disease and requiring relatively large doses for adequate control. Obviously, a steroid having greater anti-inflammatory potency and without a proportionately greater tendency for endocrine complications, or one with equal therapeutic efficacy but with fewer liabilities, would have practical advantages.

#### *Early Comparative Studies*

During 1951 and 1952, a series of clinical studies were conducted to compare the therapeutic effectiveness of hydrocortisone and cortisone in patients with rheumatoid arthritis,<sup>13-20</sup> the general results of which may be summarized as follows:

(1) The pattern of improvement resulting from the administration of the two steroids was much the same, but substantially smaller initial suppressive doses of hydrocortisone were required to produce corresponding inhibition of the disease manifestations.

(2) By comparing the effects of oral doses in the same patient, the anti-rheumatic potency of hydrocortisone was estimated to be about 50 per cent greater than that of cortisone acetate or its free ester. In other words, the average milligram dosage of hydrocortisone needed for roughly the same degree of improvement was approximately two-thirds that of cortisone.

(3) Substantially lower maintenance doses of hydrocortisone provided equal or superior rheumatic control, and, when these smaller daily amounts were used, certain endocrine complications appeared to be fewer or less pronounced,

especially psychic stimulation, salt and water retention, and excessive appetite. In a series of 44 patients whose medication was transferred directly from cortisone acetate to hydrocortisone, 31 demonstrated signs of hormone excess while receiving cortisone. Following transfer to smaller but equally effective maintenance amounts of hydrocortisone, one or more of these signs diminished or disappeared in 22 of the patients. There was lessening or correction of nervous symptoms in 13 of 15 patients, of edema in 9 of 15 patients, of excessive appetite in 9 of 16 patients, of facial mooning and/or supraclavicular fat pads in 9 of 23 patients, and of generalized obesity in 2 of 9 patients. Hypertrichosis (12 patients) and irregular glycosuria (2 patients) were unchanged.

These observations suggested that there was a greater dissociation between the desirable anti-inflammatory action and certain other undesirable physiologic effects with hydrocortisone than with cortisone, and that hydrocortisone might possess a higher therapeutic index—*i.e.*, might provide equal or greater benefits with lower levels of dosage and, at the same time, might produce fewer or less marked untoward reactions.

### *Present Study*

For this report, the results of prolonged oral hydrocortisone therapy\* in 150 consecutive patients with active peripheral rheumatoid arthritis were analyzed. As statistical results are necessarily contingent on a number of factors (including the composition of the series in relation to disease severity and duration, the therapeutic plan and objective, the method of dosage regulation, opinion as to what constitutes satisfactory response, and judgment as to the acceptability and safety of hormonal complications), some of these aspects, as applied to the present study, deserve clarification.

Each patient selected for the study first had been given a fair but unsuccessful trial on conventional conservative measures for rheumatoid arthritis. The majority of the patients suffered from more severe forms of the disease. The arthritis was graded as severe in 39 patients (26 per cent), moderately severe in 70 (47 per cent), and moderate in 41 (27 per cent). No patients with mild disease were included, as we believed that they should be managed with more conservative measures.

Our guiding therapeutic policy was to maintain as much relief as possible with doses of hydrocortisone which could be well tolerated. Complete inhibition of the disease manifestations and total functional rehabilitation were not sought unless these could be accomplished with so-called safe levels of dosage. Submaximal improvement of about 75 to 85 per cent of the pretreatment status was considered optimal for long-term administration. This policy was adopted because it was recognized that benefits from the hormone were only suppressive, not curative, and, from previous experience with cortisone, it was learned that the incidence and severity of hormonal complications were directly related to the size of the dose employed, and that their occurrence was a major factor influencing the success of long-term therapy.

Adequate or major improvement by our appraisals meant very marked, or

\* The hydrocortisone (free alcohol) used for this investigation was supplied in part through the courtesy of the Medical Department, Merck & Co., Inc., Rahway, N. J.



marked, and corresponded to over-all improvement of approximately 75 per cent or more, as compared to the pretreatment status. It should be emphasized that improvement designated as inadequate was at least worth while, or treatment would have been discontinued. Many patients whose improvement was so labeled were not displeased with their results, although most of them would have welcomed greater relief if it could have been provided safely.

The following plan of dosage, consisting of three stages, was used:

(1) *Initial suppressive doses.* As a rule, total daily dosages were employed at the beginning: for severe cases, about 50 to 70 mg.; for moderately severe cases, about 40 to 60 mg.; for moderate cases, about 40 to 50 mg. These amounts were continued until the clinical manifestations were satisfactorily suppressed, ordinarily for one to three weeks.

(2) *Reduction of dosage.* Dosage was then gradually lowered in stepdown fashion, reductions of 5 mg. being made every 7 to 14 days or, sometimes, even more slowly. The smallest total daily amount that would control the manifestations adequately, not necessarily completely, and that could be safely tolerated, was considered as the maintenance dose.

(3) *Maintenance dosage.* Maintenance doses ordinarily ranged from 45 to 65 mg. in severe cases, from 40 to 50 mg. in moderately severe cases, and from 25 to 40 mg. in moderate cases. Once established, maintenance therapy was continued without interruption, the dosage being manipulated from time to time to accommodate shifts in disease activity or to control adverse reactions. Routinely, the total daily requirement was taken in four divided doses, with a portion ingested at mealtimes and at bedtime. Dosage adjustments were usually made by small increments or decrements of 5 or 10 mg. at a time. Large "booster" doses were rarely needed.

Minor endocrine side effects, such as slight to moderate facial mooning or hypertrichosis, slight peripheral edema, or irregular glycosuria, were usually looked upon as acceptable annoyances and not as reasons for stopping treatment.

Adjunctive measures such as regulated rest, avoidance of emotional stress, physiotherapy, and a well-balanced diet, relatively low in salt and with caloric restriction, when indicated, were prescribed simultaneously. Salicylates were used regularly or irregularly by some patients and intra-articular injections of hydrocortisone acetate were occasionally employed in others to suppress exacerbations in one or two joints.

### *General Results*

As time permits presentation of only a part of the data compiled, an attempt will be made to answer a few general but pertinent questions.

(1) *What percentage of patients started on hydrocortisone therapy discontinued the hormone and for what reasons?* The 150 patients in this study were under observation for periods of 9 to 36 months from the beginning of therapy. More than one third of them (37 per cent) were followed for two years or longer. Treatment was stopped in 24 patients (16 per cent), and the remaining 126 (84 per cent) were still taking the steroid at the time of the analysis. Reasons for discontinuance were as follows: Insufficient clinical response to warrant

TABLE 1

GENERAL DATA ON 150 CONSECUTIVE RHEUMATOID ARTHRITIC PATIENTS BEGUN ON HYDROCORTISONE THERAPY

(At Analysis—9 to 36 Months After Instituting R)

Patients still on therapy.....	126 (84%)
Patients therapy discontinued.....	24 (16%)
Reasons for discontinuing therapy:	
(1) Insufficient clinical result.....	4 (2.7%)
(2) Major complication from hydrocortisone.....	2 (1.3%)
(3) Death from causes unrelated to hydrocortisone.....	5 (3.3%)
(4) Complication unrelated to hydrocortisone.....	1 (0.7%)
(5) Disease remission.....	12 (8%)
Complete.....	9 (6%)
Partial.....	3 (2%)

further administration in 4 (2.7 per cent); major complications attributable to the hormone in 2 (1.3 per cent); complication unrelated to hydrocortisone in 1 (0.7 per cent); death from extraneous causes in 5 (3.3 per cent); and complete or nearly complete remission in 12 (8.0 per cent) patients (TABLE 1).

(2) *What over-all results may be achieved from prolonged hydrocortisone therapy?* At the time of analysis, improvement was considered to be adequate or satisfactory in 59 per cent of patients and less than satisfactory in 41 per cent (FIGURE 1). Inadequate degrees of improvement were noted in 62 patients for one or more reasons: 37 (62 per cent) failed to respond satisfactorily to reasonable-sized doses from the beginning of treatment; 13 (22 per cent) were controlled well at first, but later became relatively refractory to the hormone; 37 (62 per cent) developed hormonal side reactions which prohibited the use of effective doses; and 5 (8 per cent) presented miscellaneous other reasons for limited improvement. Female patients fared almost as well as male patients. Inadequate improvement was recorded in 42 per cent of the former and in 39 per cent of the latter.

(3) *What are the chief factors which influence the success of therapy?* Aside from

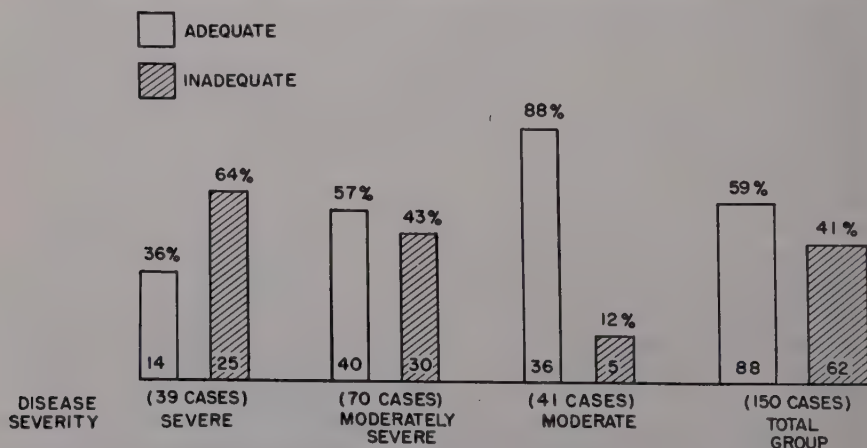


FIGURE 1. Hydrocortisone therapy: improvement in relation to disease severity.

recognized correctible mistakes in management (*i.e.*, poor selection of patients, inadequate supervision, improper dosage regulation, neglect of appropriate rest and simple complementary measures, avoidance of trauma, *etc.*), which may lead to failure, it was apparent from this study that therapeutic results bear important relationship to two factors—the severity or activity of the disease and the duration of arthritis prior to treatment.

At the time of analysis, improvement was graded as adequate in 36 per cent of patients with severe, 57 per cent of patients with moderately severe, and 88 per cent of patients with moderate disease (FIGURE 1). In general, the same problem pertained as with cortisone: For satisfactory control the more severe cases all too often required excessive doses—doses which could not be tolerated or which were considered unsafe for long-term administration. Surely, statistical results would have been more favorable had the series contained a larger percentage of moderate and some mild cases. As might be expected, the remission rate was greater in patients with moderate disease (14.5 per cent) than in those whose arthritis was moderately severe (7.1 per cent) or severe (0.025 per cent).

Percentagewise, results were most favorable when the arthritis was of relatively recent origin. Interestingly, the crucial point was about two years and, thereafter, as disease durations lengthened, the proportion of adequate responses lessened progressively (FIGURE 2). Not surprising was the finding that the remission rate was decidedly lower in patients whose disease had been established more than two years (4 per cent) than when the duration was two years or less (22 per cent).

(4) *What deterioration of improvement may be expected as treatment is prolonged?* Analyses made at intervals during the period of observation revealed, as anticipated, that as treatment was continued over the months, the number of patients showing adequate improvement became smaller. The figures (FIGURE 3) may be confusing unless it is understood that patients were added to the series as the study progressed and that others were dropped from time to time because of remission, insufficient benefit, or other reasons. The disease was satisfactorily restrained in 72 per cent of patients at the end of six months,

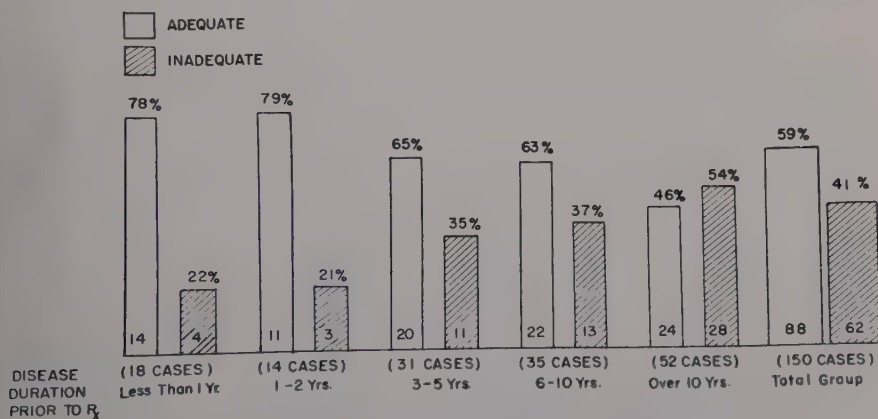


FIGURE 2. Hydrocortisone therapy: improvement in relation to disease duration.

but this percentage declined to 59 per cent at 18 months. At 24 months, and later, the number demonstrating satisfactory control rose to around 70 per cent. This increase is probably explained by the fact that the majority of patients who developed refractoriness or unacceptable complications discontinued the hormone within a two-year period.

(5) *What influence does long-term hydrocortisone therapy have on the progress of the disease?* Among the 150 patients, 50 of them (33 per cent) showed evidence of disease progression during the observation period. Functional capacity was not altered significantly by advancement of the arthritis in 19 of the patients, but it was altered in the remaining 31 patients. As might be anticipated, the ability of steroid therapy to restrain disease progress varied indirectly with the severity or activity of the rheumatoid arthritis. Progression was noted in 44 per cent of severe cases, 34 per cent of moderately severe cases, and in 22 per cent of moderate cases (FIGURE 4). An unexpected finding, however, was the lack of correlation between the frequency of disease progression and the duration of the arthritis prior to therapy in this series (FIGURE 5).

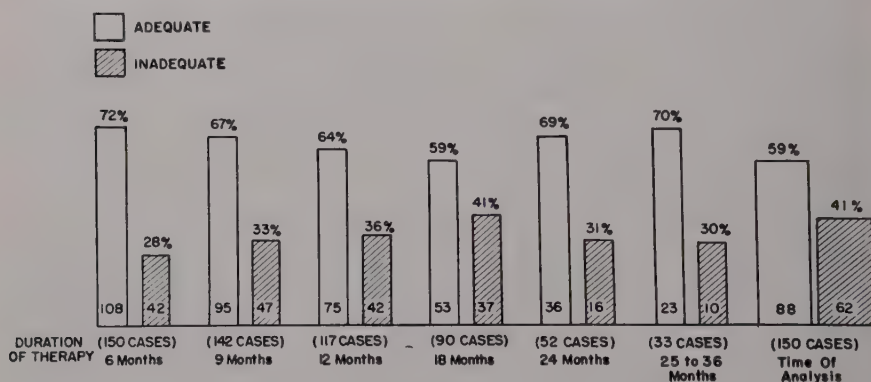


FIGURE 3. Hydrocortisone therapy: improvement in relation to duration of treatment.

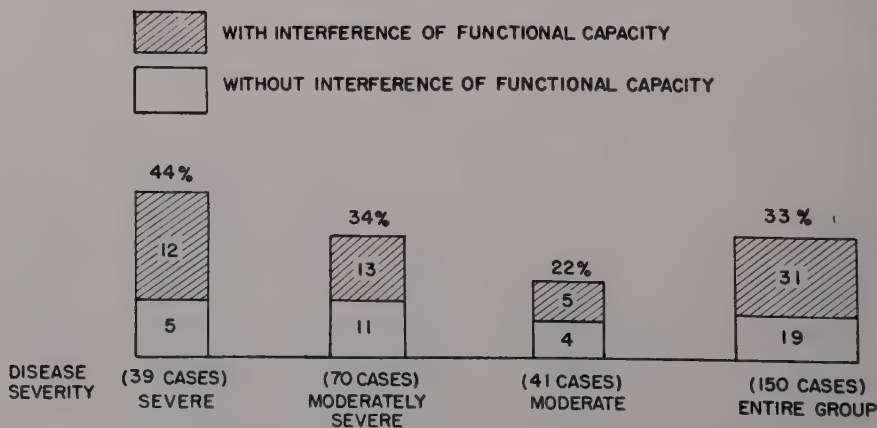


FIGURE 4. Hydrocortisone therapy: disease progression in relation to disease severity.



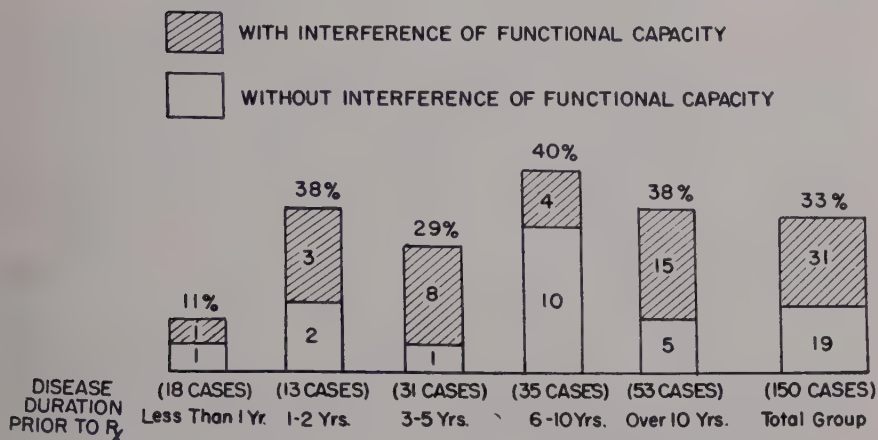


FIGURE 5. Hydrocortisone therapy: disease progression in relation to disease duration.

It is impossible to judge with full assurance what influence hydrocortisone therapy may have exerted on the natural course of the disease. There are no statistical data available with which to compare the incidence or rate of disease progression in untreated or conservatively treated patients in a series of similar composition. Furthermore, the periods of observation in the present study were relatively short (9 to 36 months) in respect to the average span of the disease. When, however, consideration is given to the fact that the majority of patients (73 per cent) suffered from severe or moderately severe forms of rheumatoid arthritis, and also to the fact that they had already failed to respond satisfactorily to conservative management, the incidence of disease progression seems smaller than might have been projected for a similar untreated series. The impression was gained that in cases amenable to hydrocortisone, advancement of the rheumatoid process may be retarded or halted during the period of steroid administration and, perhaps, more frequently than heretofore considered.

(6) *How satisfactory is hydrocortisone as a long-term treatment agent for rheumatoid arthritis?* Orally-administered hydrocortisone must be regarded as a very valuable agent in the management of selected cases of rheumatoid arthritis. It promotes a high rate of immediate therapeutic response and, with apparently safe and relatively low maintenance doses, it is capable of providing continuous, adequate control of the rheumatic manifestations in a large proportion of cases. Sixty per cent of patients in the present series, whose arthritis was unsuccessfully regulated by measures other than steroid therapy, were held in major improvement by the drug during observation periods ranging from 9 to 36 months. Though benefits were less than desired, varying amounts of helpful relief and enhanced functional capacity were furnished to the majority of patients in the remaining 40 per cent.

Nevertheless, hydrocortisone is far from an ideal therapeutic agent for rheumatoid arthritis. Apart from its main deficiency, that of having suppressive rather than curative action, it has, like cortisone, many serious short-

comings. Among these deficiencies may be listed: (1) the intervention of hormonal side effects which serve to limit dosage and, frequently, satisfactory management, especially in patients with more severe or long-established disease whose daily requirements for the drug are large; (2) the tendency to aggravate certain coexisting pathologic conditions, which sets up contraindications for its use; (3) the development of relative refractoriness in some patients after prolonged administration, with resultant deterioration of improvement; and (4) failure to prevent progression of the disease during treatment in at least one third of the patients.

Thus, until a cure for rheumatoid arthritis is found, there is need for an agent which, on systemic administration, will suppress the disease more successfully over long periods and in a higher proportion of patients. A steroid with wide disparity between its anti-inflammatory power and its tendency to produce unwanted effects would partially fulfill this need. That the preparation of such a compound is possible is suggested by a number of encouraging new developments, some of which are presented in this monograph.

### References

1. HECHTER, O. 1950. Characterization of corticosteroids released from perfused cow adrenals. *Federation Proc.* **9**: 58.
2. HECHTER, O. *et al.* 1951. Nature and biogenesis of adrenal secretory product. *In* Recent Progress in Hormone Research. **6**: 215. Academic Press. New York, N. Y.
3. REICH, H., D. H. NELSON & A. ZAFFARONI. 1950. Isolation of 17-hydroxycorticosterone from blood obtained from adrenal veins of dogs. *J. Biol. Chem.* **187**: 411.
4. SAVARD, K., W. J. KOLFF & A. C. CORCORAN. 1952. Corticoids of peripheral blood. *Endocrinology*. **60**: 366.
5. BUSH, I. E. 1951. Hormones in adrenal venous effluent. *J. Physiol.* **112**(Proc. 10).
6. CONN, J. W., H. L. LAWRENCE & S. S. FAJANS. 1951. The probability that Compound F (17-hydroxycorticosterone) is the hormone produced by the normal adrenal cortex. *Science*. **113**: 713.
7. JACOBSEN, R. P. & G. PINCUS. 1951. The chemistry of adrenal steroids. *Am. J. Med.* **10**: 531.
8. PINCUS, G. 1949. Adrenal Cortex Function in Stress. *Trans. 1st Conf. Josiah Macy, Jr. Found.*, New York, N. Y.
9. MASON, H. L. 1950. Urinary excretion of steroids during administration of ACTH: 168. *In* Clinical ACTH Conf. Proc. 1st Meet. J. R. Mote, Ed. Blakiston. Philadelphia, Pa.
10. INGLE, D. J. & M. H. KUIZENGA. 1945. The relative potency of some adrenal cortical steroids in the muscle-work test. *Endocrinology*. **36**: 218.
11. PABST, M. L., R. SHEPPARD & M. H. KUIZENGA. 1947. Comparison of liver-glycogen deposition and work performance tests for bio-assay of adrenal cortex hormones. *Endocrinology*. **41**: 55.
12. HENCH, P. S., E. C. KENDALL, C. H. SLOCUMB & H. F. POLLEY. 1950. Effects of cortisone acetate and pituitary ACTH on rheumatoid arthritis, rheumatic fever and certain other conditions; a study in clinical physiology. *Arch. Internal Med.* **85**: 545.
13. BOLAND, E. W. 1952. Antirheumatic effects of hydrocortisone (free alcohol), hydrocortisone acetate, and cortisone (free alcohol) as compared with cortisone acetate: results from oral administration in patients with rheumatoid arthritis. *Brit. Med. J.* **1**: 559.
14. BOLAND, E. W. & N. E. HEADLEY. 1952. Compound F used orally in patients with rheumatoid arthritis. *J. Am. Med. Assoc.* **148**: 981.
15. BOLAND, E. W. 1952. Rheumatoid arthritis: experiences with hydrocortisone (free alcohol) and hydrocortisone acetate. *Calif. Med.* **77**: 1.
16. BOLAND, E. W. 1952. Hydrocortisone (Kendall's Compound F): experiences with the free and acetated forms in rheumatoid arthritis. *J. Am. Pharm. Assoc., Prac. Pharm. Ed.* **13**: 540.

17. BOLAND, E. W. 1952. Clinical use of cortisone, hydrocortisone and corticotropin. J. Am. Med. Assoc. **150**: 1281.
18. BOLAND, E. W. 1953. Systemic use of hydrocortisone. The Merck Rept. **62**: 12.
19. BOLAND, E. W. 1953. Hydrocortisone administered orally in rheumatoid arthritis. Ann. Rheumatic Diseases. **12**: 125.
20. BOLAND, E. W. 1954. Oral hydrocortisone in the treatment of rheumatoid arthritis. Med. Clinics N. Amer. **38**: 337.

## METABOLIC EFFECTS OF METACORTANDRALONE AND METACORTANDRACIN

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When we originally presented our observations on the effects of metacortandralone and metacortandracin before the Interim Session of the American Rheumatism Association on November 4, 1954, we did not know the chemical structure of these steroids. We did know that the compounds were synthetic, crystalline steroids with characteristic infrared absorption spectra. Animal experiments had revealed that these steroids were nontoxic and that they induced eosinophilic depletion, liver glycogen deposition, and thymic involution.\* We then decided to subject the new steroids to clinical trial in patients with rheumatoid arthritis in order to assess their anti-inflammatory and antirheumatic properties.

We are now able to present the structural formulae of metacortandralone and metacortandracin† (FIGURE 1). Metacortandralone is delta 1,4-pregnadiene-11 beta, 17 alpha, 21-triol-3, 20-dione. It is an analog of hydrocortisone, and differs from it by having an unsaturated bond at carbon 1. Metacortandracin resembles metacortandralone except that it has a ketone instead of an hydroxyl group at carbon 11. It is an analog of cortisone and differs from it by having an unsaturated bond at carbon 1.

*Clinical Effects.* In a previous report,<sup>2</sup> the results of our studies on the first seven patients with rheumatoid arthritis were published. To date, we have observed a total of 15 patients. The distribution of patients as to sex, age, duration, and stage of arthritis is given in TABLE 1. In every case, the arthritis was active and progressive, and had failed to respond to the therapeutic measures listed in TABLE 2.

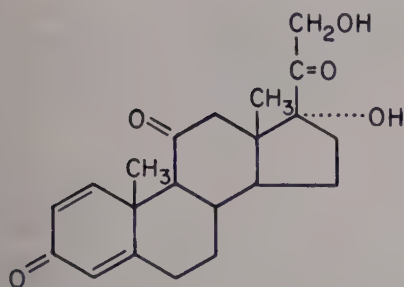
The new steroids were administered orally every six or eight hours in initial doses of 30 mgm. daily for most patients. Every patient but two exhibited marked subjective and objective, articular and generalized improvement. Decidedly favorable response began within the first 24 hours of medication. Subjective improvement reached a peak by the third day, and objective improvement by the end of the third week. As antirheumatic agents, both metacortandralone and metacortandracin were found to be three to four times more potent than hydrocortisone or cortisone. Preliminary observations over a period of five months indicate that this enhanced potency is not accompanied by a corresponding increase in the frequency or severity of undesirable side effects. These steroids are not, however, free of side effects, as will be described later.

The duration of administration of the new steroids in this series varied from four weeks to five months. The clinical results are given in TABLE 3. The two patients who showed only minor improvement had advanced arthritis

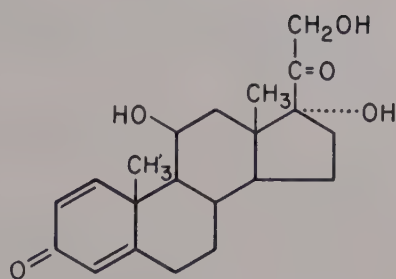
\* We are indebted to the research team of the Schering Corporation, Bloomfield, N. J., for this information.<sup>1</sup>

† The formulae were given to us by the Schering Corporation, on January 25, 1955.





METACORTANDRACIN



METACORTANDRALONE

FIGURE 1

TABLE 1  
COMPOSITION OF SERIES  
15 Patients with Rheumatoid Arthritis

Females.....	12	Below age 40.....	5
Males.....	3	Between 40 and 60.....	7
		Above age 60.....	3
Duration of disease		Stage of arthritis	
2 to 5 yr.....	7	Early (stage 1).....	0
6 to 10 yr.....	4	Moderate (stage 2).....	5
11 to 15 yr.....	3	Advanced (stage 3).....	6
Over 15 yr.....	1	Far advanced (stage 4).....	4

with a large component of irreversible changes. The nature of the clinical response following administration of the steroids and evidence of antirheumatic and anti-inflammatory properties of the compounds are presented elsewhere.<sup>2</sup>

As therapy is prolonged, some patients exhibit a flare of the arthritis and require increased amounts of the hormone in order to maintain the improvement initially achieved. The recurrence of clinical manifestations on maintenance therapy is accompanied by corresponding laboratory changes. FIGURE 2 illustrates such a case. The dose of metacortandracin was increased from 20 to 30 mgm. daily on about the 100th day of therapy. This was done because, at this time, pain, swelling, tenderness, and stiffness in the involved joints, which had initially subsided almost completely, returned to pretreatment level. The erythrocyte sedimentation rate (ESR) increased, and the C-reactive protein reappeared. The hematocrit and the cholesterol concentration of the serum, which had increased initially during therapy, now diminished on continued medication, although they did not return to pretreatment values. When the dose was increased, the signs and symptoms of arthritis again subsided to a marked degree. The C-reactive protein disappeared, but the ESR, hematocrit, and cholesterol showed little change.

*Effect on electrolytes.* Weekly determinations of the concentration in the

TABLE 2  
THERAPY PREVIOUSLY ADMINISTERED WITH UNSATISFACTORY RESULTS

	No. of Patients
Cortisone, hydrocortisone, corticotropin.....	12
Gold compounds.....	9
Phenylbutazone.....	6
Salicylates only.....	3

TABLE 3  
THERAPEUTIC RESULTS WITH METACORTANDRALONE OR METACORTANDRACIN

Remission during therapy*	8
Major improvement.....	5
Minor improvement.....	2
No improvement.....	0

\* No objective signs of active arthritis, normal or near-normal erythrocyte sedimentation rate, negative C-reactive protein, and normal albumin-globulin ratio.

blood of sodium, potassium, calcium, phosphorus, chlorides, and carbon dioxide before and during metacortandralone or metacortandracin administration disclosed no significant changes. Sodium and potassium balance studies done on two patients receiving 30 or 50 mgm. of metacortandralone daily showed no retention of sodium and no increased loss of potassium (FIGURE 3).

*Protein metabolism.* Nitrogen balance studies were done on a 16-year-old Negro male patient with rheumatoid arthritis before, during, between, and after three courses of metacortandralone and metacortandracin (FIGURES 3 and 4). The youth was on a constant daily dietary intake of 2800 calories containing 15.25 grams of nitrogen. During the first course, 30 mgm. of metacortandralone were given daily for 12 days. Urinary nitrogen excretion did not increase. (In FIGURE 3, only urinary nitrogen is included, although both fecal and urinary nitrogen determinations were done.) During the second course, 50 mgm. of metacortandralone was given daily for 24 days. No change in nitrogen excretion occurred for about the first 10 days. Thereafter, however, urinary nitrogen excretion increased from the mean control level of 12.8 gm. to a level averaging 16 gm. during the third and fourth six-day periods of drug administration. The steroid was then abruptly discontinued and withheld for 18 days. During this phase (FIGURE 4), the patient was in marked positive nitrogen balance (rebound). When 50 mgm. of metacortandracin was given daily for 30 days, urinary nitrogen excretion increased approximately 60 per cent, although the balance became only barely negative. When the steroid was again discontinued, urinary nitrogen excretion decreased sharply and the balance again became markedly positive. It should be noted in FIGURE 4 that body weight increased in association with periods of marked positive nitrogen balance occurring during the control phases which preceded and followed steroid administration. Urinary creatinine is shown on the chart to indicate its relative day-to-day constancy, thus documenting the completeness of 24-hour urine collections.\*

*Serum protein fractions.* During treatment with metacortandralone and

\* We are indebted to G. Donald Whedon, who conducted the nitrogen balance studies.

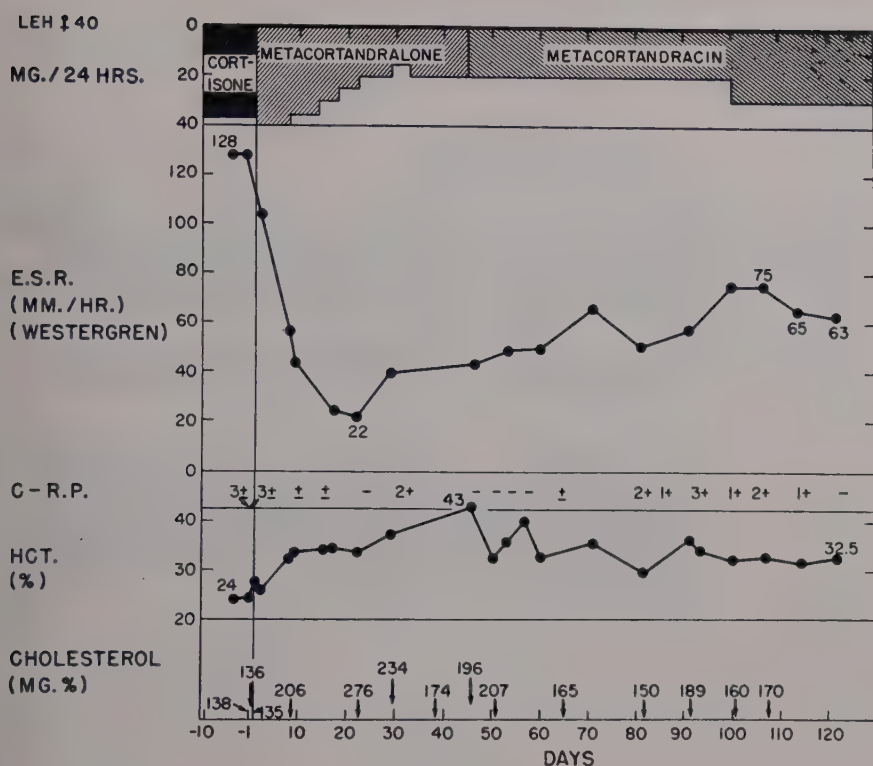


FIGURE 2

metacortandracin, these patients showed a tendency toward a rise in serum albumin and a fall in serum globulin. The rise in albumin occurred in 13 of the 15 patients, and averaged 0.7 gm. per cent. A fall in globulin occurred in 11 of the 14 patients and averaged 0.8 gm. per cent. The duration of therapy at the time of maximum change in the serum proteins showed considerable variation, but was usually four to six weeks.

*Carbohydrate metabolism.* Determinations of fasting blood glucose levels were done frequently on every patient, and none showed a definite rise above pretreatment levels.\* Glycosuria did not appear in any case. Results of glucose tolerance tests done in six patients before and during metacortandralone and metacortandracin administration are recorded in TABLE 4.

The blood glucose concentration following glucose ingestion was higher during steroid administration than during control periods, in all six patients tested. It should be noted that in case No. 8, an abnormal curve was present even before treatment. In four of the remaining five cases (Nos. 1, 2, 9, and 13) the peak concentration exceeded 150 mg. per cent. In two of these four cases, the glucose concentration of the blood at two hours was above 100 mg. per cent. Thus, according to the criteria of Fajans and Conn,<sup>4</sup> two of the five

\* Nelson modification of Somogyi method was used.<sup>3</sup> All patients were on constant diet before and during steroid administration.

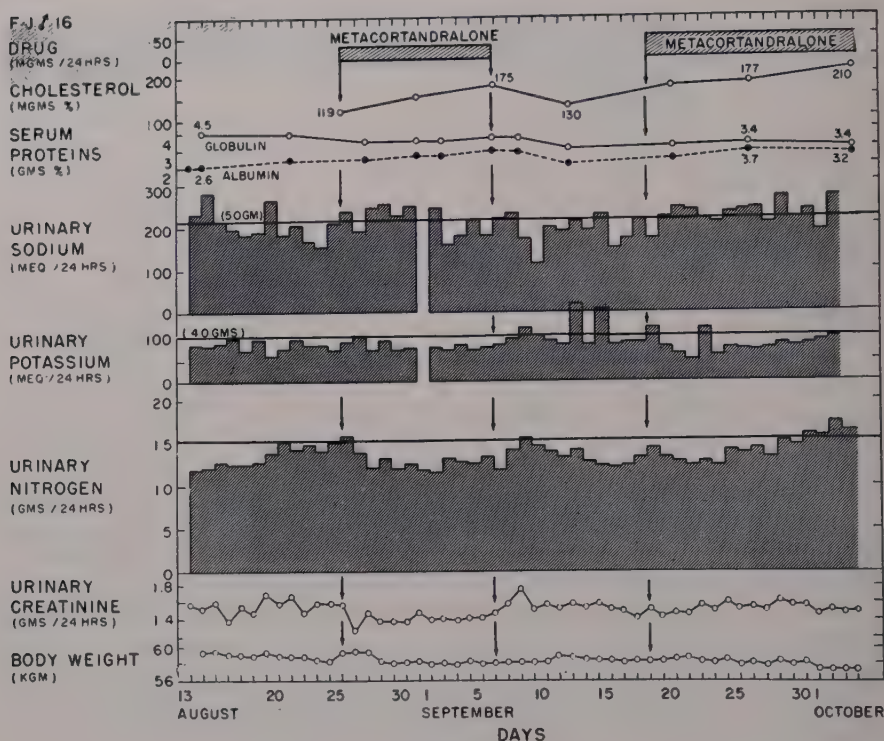


FIGURE 3

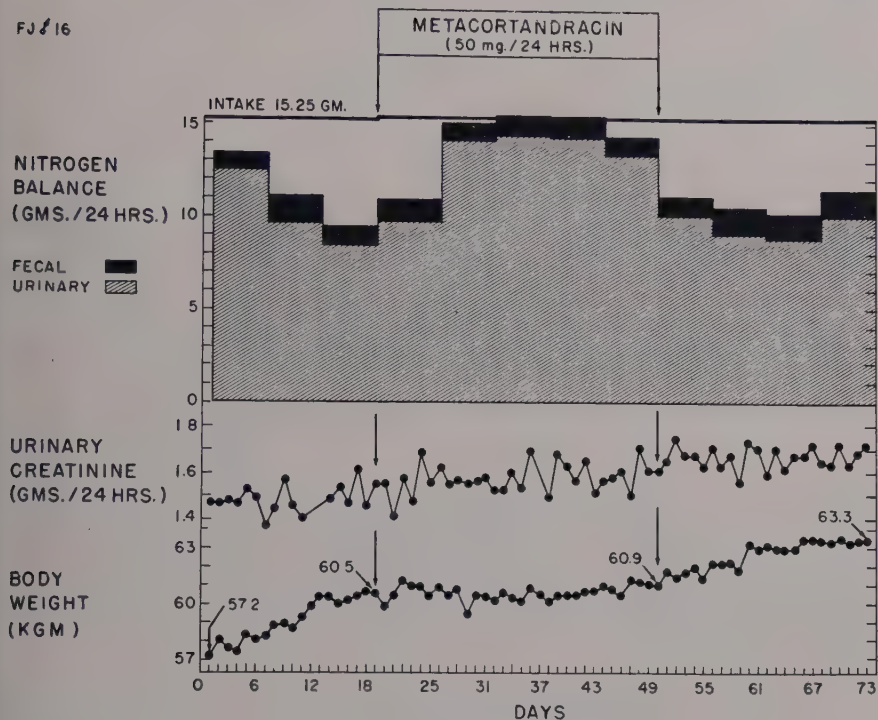
patients studied (cases Nos. 9 and 13) developed diminished carbohydrate tolerance as a result of metacortandracin administration.

*Cholesterol metabolism.* TABLE 5 lists the changes in serum cholesterol during administration of metacortandralone or metacortandracin. It will be noted that a rise in serum cholesterol occurred in every patient in this series during treatment. This rise averaged 82 mgm. per cent, usually reaching a maximum during the fourth or fifth week of therapy. The increase was in the total cholesterol of serum. Since no change in the esterified fraction occurred, a fall in the percentage of esters resulted.

*Hormonal effects.* Oral administration of metacortandralone resulted in uniform, prompt, and significant fall (over 50 per cent) in circulating eosinophils and suppression of urinary 17-ketosteroids. When the steroid was discontinued, the eosinophils rose promptly and reached premedication levels in one week. Excretion of 17-ketosteroids increased on the second day after metacortandralone was stopped, but failed to reach pretherapy levels during the following week (FIGURE 5). A relation was observed between the level to which eosinophils fell and the dose of metacortandralone.

The 17-ketosteroid excretion in a 32-year-old female patient with rheumatoid arthritis who was given 30 mgm. of metacortandralone daily decreased from 8.3 mgm. per 24 hours before medication to 3.8 on the first day and 1.8 mgm.





FIGURE

on the fifth day. In a 16-year-old Negro male with the same disease, given the same dosage of metacortandralone, the 17-ketosteroid excretion fell from 8.0 to 4.5 mgm. per day. Reduction of urinary 17-ketosteroid indicates adrenocortical suppression resulting from reduced secretion of adrenocorticotropin by the anterior pituitary.

*Undesirable side effects.* TABLE 6 lists the unwanted effects that occurred among a total of the 15 patients treated. A duodenal ulcer was detected by X ray in one patient during the 12th week of treatment. This patient was not subjected to X ray study before treatment. She had no clinical symptoms or signs of ulcer at any time. After the ulcer was detected, metacortandracin was continued and aluminum hydroxide was administered. X ray of the stomach and duodenum repeated three weeks later revealed that the ulcer had healed. In a second patient, X ray studies of the duodenum made immediately before therapy was begun showed no ulcer. Radiograms repeated during the fourth week of therapy (30 mgm. daily of metacortandracin) disclosed a duodenal ulcer. The patient was transferred to another hospital before X ray studies could be repeated. Radiographic examinations done on the other 13 patients in the series were normal.\* Urinary uropepsin excretions, done by Mirsky's method,<sup>5</sup> were determined in three patients by Doctor Lee Hershenson prior to,

\* Following presentation of this paper, a third patient in this series was found to have developed radiologic evidence suggestive of a duodenal ulcer, after having had normal gastrointestinal X rays earlier.

TABLE 4  
GLUCOSE TOLERANCE TESTS\*

Case No.†	No. days of therapy	Total amount of steroid given (mg.)	FBS	30'	60'	120'	180'
1	0 11	0 330	71 79	147 171	66 115	59 68	62 —
2‡	8 10 0	240 500 0	80 89 71	159 212 99	118 132 82	61 87 89	69 85 51
8	0 15	0 450	91 89	126 155	203 232	166 173	93 78
9	0 20	0 600	66 90	122 170	139 217	83 110	45 71
12	0 30	0 900	86 63	123 131	116 140	106 86	65 60
13	0 31	0 870	69 80	126 168	113 190	104 102	47 48

\* One gram of glucose per kilogram of body weight was administered orally to the fasting patient.

† In case 1, metacortandralone only was administered; in case 2, metacortandralone and metacortandracin were given; in the remaining cases, metacortandracin only was prescribed.

‡ The control test was done on this patient *after* the steroid was discontinued.

FBS = Fasting blood sugar.

TABLE 5  
CHANGES IN SERUM CHOLESTEROL\* DURING METACORTANDRALONE AND METACORTADRACIN ADMINISTRATION

Case No.	Pretherapy level mg. per 100 cc.	Maximum level during therapy mg. per 100 cc.	Day of therapy on which maximum level occurred	Total steroid given by this day mg.
2 (30 mg./day)	119	175	12	360
2 (50 mg./day)	130	210	16	800
3	162	299	26	1,000
4	138	276	21	715
5	123	170	35	820
6	303†	411	28	1,475
8	205	269	29	960
9	124	259	22	665
10	185	226	41	1,230
11	153	191	39	765
12	190	256	46	1,470
13	144	238	32	895
14	215	296	19	415
15	193	247	47	955
Mean.....	170	252	30	895

\* Schoenheimer-Sperry method was used.

† This patient had coexisting nephrosis unrelated to rheumatoid arthritis or steroid therapy.

and again during, steroid therapy. No change in uropepsin excretion occurred in any of these patients, including the one who developed duodenal ulcer, as described above.

Mental changes noted in two of the three patients were mild and evanescent.

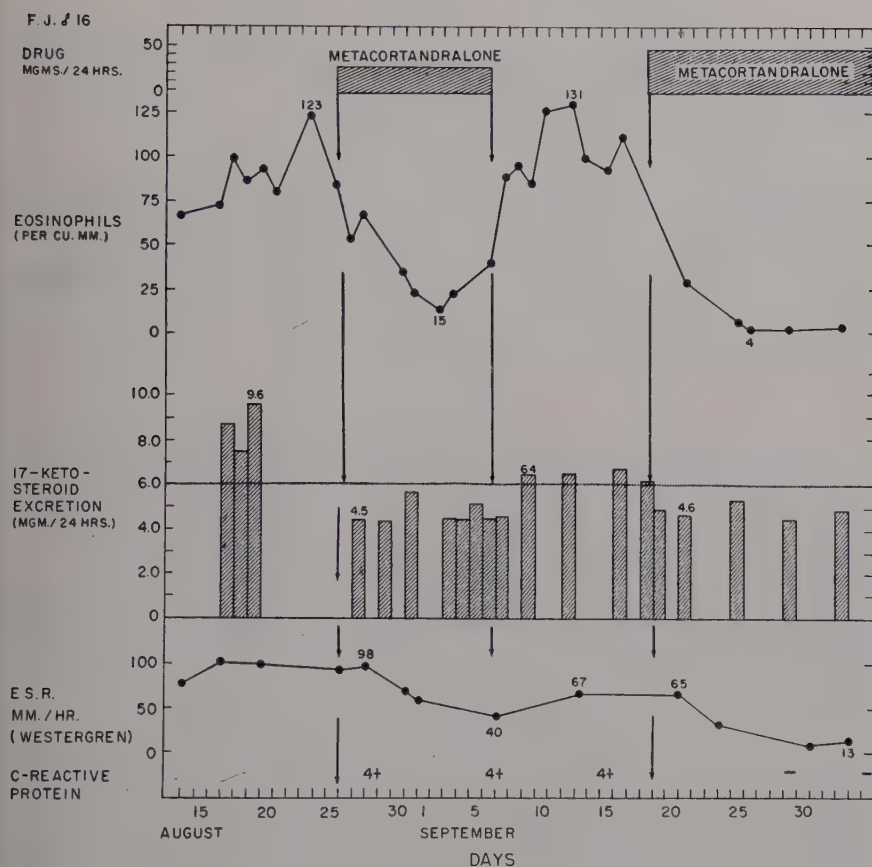


FIGURE 5

TABLE 6  
UNDESIRABLE SIDE-EFFECTS NOTED IN A SERIES OF 15 PATIENTS

Nature	No. of patients
Epigastric discomfort (transitory).....	6
Sleeplessness and restlessness.....	5
Increased sweating and flushes (menopausal).....	5
Facial rounding.....	4
Hirsutism.....	3
Weakness and fatigue.....	3
Mental changes.....	3 <sup>†</sup>
Ulcer, duodenal.....	2 <sup>†</sup>
Acneiform eruption.....	2

\* One of these patients developed psychosis (see text).

† Since this paper was presented, a third patient developed duodenal ulcer (see text).

In a third patient, a 32-year-old single Negro woman, however, a psychotic depression developed during the fourth week of metacortandracin administration. She had been receiving 30 mgm. daily. It is noteworthy that this patient had been frequently observed for several months by Doctor Ernest Kahn, a staff psychiatrist, one year before treatment. Doctor Kahn had found her to be a "diffident and phlegmatic, of low intelligence, and mildly, chronically depressed." The diagnostic impression was "passive-aggressive personality with depressive reaction." When mental changes occurred, during therapy, the steroid was withdrawn in decrements of 10 mgm. per day. There was no relapse of the arthritis or systemic signs of withdrawal. The psychosis did not improve, however, and the patient was transferred to a mental institution 10 days after discontinuance of therapy.

### Summary

Metacortandralone and metacortandracin are synthetic crystalline steroids that are four times more potent than cortisone or hydrocortisone as anti-rheumatic agents. Administration of these new steroids is followed by immediate and marked improvement in patients with rheumatoid arthritis, including those who have stopped responding to cortisone or hydrocortisone. The steroids are not free, however, from considerable side effects.

Administration of these compounds is followed by a prompt fall in circulating eosinophils and by a significant reduction in urinary 17-ketosteroid excretion.

The effects on carbohydrate, protein, cholesterol, and electrolyte metabolism are described.

### References

1. HERZOG, H. O. *et al.* 1955. New anti-arthritic agents. *Science*. **121**: 176.
2. BUNIM, J. J., M. M. PECHET & A. J. BOLLET. 1955. Studies on metacortandralone and metacortandracin in rheumatoid arthritis. *J. Am. Med. Assoc.* **157**: 311.
3. NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **153**: 375.
4. FAJANS, S. S. & J. W. CONN. 1954. An approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone. *Diabetes*. **3**: 276.
5. MIRSKY, A., S. BLOCK, S. OSHER & R. H. BROH-KAHN. 1948. Uropepsin excretion by man. *J. Clin. Invest.* **27**: 818.

### Discussion of the Paper

DOCTOR MAURICE M. PECHET: We have some observations on one normal patient and one Addisonian which would supplement some of the work done by Doctor Bunim in the Arthritic Institute. A 53-year-old normal woman was hospitalized for a diverticulum of the esophagus. She received 70 mg. of metacortandralone a day. To begin with, there was a slight retention because she was not in equilibrium before starting on the 70 mg. However, she had a diuresis equivalent to about 40 mEq. of sodium and probably at the end of about the ninth day was beginning to escape. When the dose was cut to 30 mg. of metacortandralone, the diuresis was less marked.

This is in marked contrast to what happens with 150 mg. a day of hydrocortisone. There was this marked retention of sodium as indicated by the tremendous increase in weight, of almost 3 kg. On stopping the drug there was a marked diuresis of sodium up to 237 mEq. of sodium the first day, and



345 mEq. the second day. It is probable that if she could have been maintained longer on hydrocortisone, she might have begun to diurese. As for the urinary potassium, the characteristic diuresis in the first 24 hours on hydrocortisone occurred. There is a marked effect on urinary nitrogen with Metacorten. There is a loss of nitrogen which progressively increases up to a point where the patient is losing a considerable amount daily. At a 30 mg. dose in terms of urinary nitrogen, there is a very slight loss, but if nitrogen intake is superimposed, the patient is in slight negative balance and about the same situation occurs on 150 mg. of hydrocortisone.

A 38-year-old Addisonian female patient was put on an intake of 250 mEq. of sodium a day. With 10 mg. a day of Meticorten there was a slight sodium diuresis, in marked contrast to what happened when she was on 150 mg. of cortisone acetate. There was a marked urinary potassium diuresis in the first 24 hours on both Meticorten and cortisone acetate.

In this patient, there was a loss of urinary nitrogen which reached a maximum on the tenth day of administration, and it is evident that it is difficult to give a factor whereby one could say Meticorten is so many times more active than cortisone, because it depends on what is being measured. The effect on 17-ketosteroid excretion in a normal patient on 70 mg. showed a drop in the 17-ketosteroid excretion and a marked increase in the 17 hydroxy-corticoids.

I wish also to point out to those investigators who are employing the Porter-Silber technique, using phenylhydrazine and sulfuric acid at  $240\ \mu$  the density of Meticorten is 80 per cent greater than that of cortisone. This factor should be taken into consideration in making any calculations.

DOCTOR EMANUEL SCHWARTZ: I should like to report the effect of metacortandracin in two cases of bronchial asthma. The new steroid was more effective than either cortisone or hydrocortisone.

DOCTOR EDWARD F. BOLAND: We have given metacortandracin to 28 patients with rheumatoid arthritis over periods ranging from 3 to 11 weeks. Our studies have been entirely clinical but, so far, from the clinical aspect at least, they generally confirm those of Doctor Bunim *et al.*

Comparisons of maintenance doses required for approximately equivalent degrees of improvement indicate that metacortandracin is somewhat more than four times as potent as cortisone. The figures have averaged from 3.3 to 1 to 5.3 to 1, with an average of 4.3 to 1.

In 22 patients who were unsatisfactorily controlled on hydrocortisone, we have transferred medication to smaller doses. In each instance, greater improvement has resulted, and some have achieved adequate levels of control with a few days. Even during the short periods of observations, some of the patients have shown notable reduction of side effects such as edema, nervous reaction, and so forth.

DOCTOR RAGAN: I have been talking to quite a few so-called "rheumatologists" and I can summarize a few of their observations.

Apparently everybody agrees with Doctor Bunim on the clinical facts as regards metacortandracin. Apparently everybody agrees with him on the marked lack of sodium retention and potassium diuresis. There are small nuances as to whether it is actually sodium diuresis or not, but there is no dis-

agreement about the fact that it has three to four and maybe five times the potency of cortisone as far as antiarthritic effects are concerned.

From what I have gathered, apparently a great deal of work is being done, at the present time, to determine whether there are other differences between this and cortisone and hydrocortisone.

DOCTOR LAURENCE KINSELL: I should like to ask Doctor Ragan whether anyone has done any calcium excretion studies. Negative calcium balance is one of the complications of long-term steroid therapy.

DOCTOR RAGAN: None of the data has been reported hitherto.

DOCTOR KINSELL: I think it should be pointed out that osteoporosis is possibly more related to nitrogen loss than to calcium loss, *per se* and, if it does cause protein breakdown, it would, in all probability, cause osteoporosis.

DOCTOR RAGAN: I think there would be some difference of opinion about that, Doctor Kinsell. Doctor Shorr does not feel that way. He has been able to put patients into positive nitrogen balance without an accompanying positive calcium balance.

DOCTOR ALVIN BARACH (*College of Physicians and Surgeons, New York, N. Y.*): I should like to mention one critical difference between cortisone and Meticorten. Although the nature of the spasm that takes place in bronchial asthma and pulmonary edema is affected much the same with cortisone, in a series of 24 cases that we have studied there was this difference in patients with a pulmonary fibrosis: in six of these cases, a weight loss of four to eight pounds took place in a week in patients who had not previously had cortisone. We are not able to say this definitely, but it appears that much of this weight loss took place from the lungs. It is our impression that a patient with pulmonary fibrosis improved qualitatively to a degree not obtained by cortisone.

DOCTOR CAMERON: I should like to ask Doctor Bunim whether he has further data on the cholesterol elevation that he has reported in a number of his patients. We have observed this in a significant number of the 40-odd patients we have under treatment at the present time. Has he done fractionation of cholesterol?

DOCTOR RAGAN: He says he has not.

DOCTOR CAMERON: I should like to be informed about Doctor Bunim's 15 cases, especially the two peptic ulcers and the three with metal change, which were not defined. They seemed rather high in Doctor Boland's figures.

DOCTOR BOLAND: I cannot answer your question, but I should like to add one point about the two diabetics with rheumatoid arthritis that we have had. The reports given about our findings were different from those of Doctor Schwartz. In both of these patients, the dosage was cut to approximately one third that of hydrocortisone, and yet the insulin requirement became slightly more, not less, a point that should not be overlooked. I should like to ask Doctor Bunim a question. Of the eight patients with remission, was the continuing remission while the drug was being administered or after the drug had been stopped?

DOCTOR RAGAN: Doctor Bunim said it was remission while on therapy. I might add that we have one patient in a nephrotic phase whom we are treating who is on constant intake and constant insulin requirement, and his glucose excretion in the urine has increased prodigiously on 25 mg. of Meticorten a day.

# RATIONALE FOR HORMONE THERAPY IN RHEUMATIC FEVER\*

By Vincent C. Kelley

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The dramatic response of acute manifestations of rheumatic fever to hormone therapy, which has been observed by many,<sup>1</sup> has been attributed solely to pharmacologic action of the hormones.<sup>2</sup> This conclusion has seemed justified since several studies in patients with rheumatic disease<sup>3-10</sup> have failed to detect evidence of any consistent abnormality in pituitary-adrenal function. These studies, however, have employed indirect techniques for evaluation of this function and cannot be considered conclusive. The data to be presented here concerning circulating concentrations of pituitary and adrenal hormones, adrenal responsiveness, and the half life of hydrocortisone in patients with rheumatic fever indicate that abnormalities of pituitary-adrenal-cortical function do occur consistently in these patients and provide rationale for hormone therapy in this disease on a physiological, as well as on a pharmacological basis.

## *Plasma 17-Hydroxycorticosteroids in Patients with Rheumatic Fever*

The hormone elaborated in most abundant quantity by the adrenal cortex in man is 17-hydroxycorticosterone (hydrocortisone).<sup>11</sup> Since this compound is the principal constituent of the 17-hydroxycorticosteroid (17-OHCS) group of adrenal hormones, the determination of plasma 17-hydroxycorticosteroids by the method of Nelson and Samuels<sup>12</sup> may be considered to reflect nearly specifically the concentration of 17-hydroxycorticosterone in plasma.

TABLE 1 shows the plasma 17-OHCS concentrations, as determined by this method, in normal children and in children with various phases of rheumatic fever. In 40 normal children, the mean plasma 17-OHCS concentration was 12  $\mu$ g. per cent. In 15 children who were studied during the first week after the onset of rheumatic symptomatology, designated here as the "early acute" phase of rheumatic fever, the mean plasma 17-OHCS concentration was 23  $\mu$ g. per cent. The plasma 17-OHCS concentrations varied extremely in patients during the second week of rheumatic activity, designated here as the "transitional phase" of rheumatic fever, but the mean value in this group did not differ statistically from the mean in the control group. In patients who had been ill with rheumatic fever longer than two weeks, however, designated here as having "well-established active" rheumatic fever, the mean plasma 17-OHCS concentration, 5.9  $\mu$ g. per cent, was significantly lower than that in the control group. In the total group of 58 patients with untreated active rheumatic fever, no patient who had been ill less than one week had a plasma 17-OHCS concentration as low as the mean value in the control group, and no patient who had been ill more than five weeks had a plasma 17-OHCS concentration as high as the mean value in the control group.<sup>13</sup>

\* The work reported here was supported in part by grants-in-aid from the National Heart Institute, National Institutes of Health (HG-911) Bethesda, Md.; the Helen Hay Whitney Foundation, New York, N. Y.; the Life Insurance Medical Research Fund, New York, N. Y.; the American Heart Association, New York, N. Y.; the Institute for the Study of Analgesic and Sedative Drugs, Elkhart, Ind.; the A. H. Robins Co., Richmond, Va.; and the Upjohn Company, Kalamazoo, Mich.

TABLE 1  
PLASMA 17-HYDROXYCORTICOSTEROID CONCENTRATIONS IN PATIENTS WITH VARIOUS PHASES  
OF RHEUMATIC FEVER

Group	No.	17-Hydroxycorticosteroids ( $\mu\text{g } \%$ )		
		Mean S.E.M.		(p vs. Controls)
Controls.....	40	12.0	$\pm 1.29$	
"Early acute" R. F.....	15	23.1	$\pm 1.49$	< .01
"Transitional phase" R. F.....	12	11.8	$\pm 2.01$	> 0.5
"Well-established active" R. F.....	31	5.9	$\pm 0.93$	< .01
Chorea.....	27	5.7	$\pm 0.86$	< .01
Inactive R. F.....	87	8.3	$\pm 0.61$	< .01

TABLE 2  
HALF LIFE OF HYDROCORTISONE IN RHEUMATIC FEVER PATIENTS

Group	No.	Age	Half life (minutes)	
			Range	Mean
Active R. F.....	7	5-15	98-199	136
Normal.....	5	7-14	60-98	83

The plasma 17-OHCS concentrations in patients with Sydenham's chorea also were low, the mean value in this group of patients, 5.7  $\mu\text{g. per cent}$ , being significantly lower than that in the control group. Likewise, in patients with inactive rheumatic fever, the mean plasma 17-OHCS concentration, 8.3  $\mu\text{g. per cent}$ , was significantly lower than that in the control group. These low concentrations of 17-OHCS in patients with inactive rheumatic fever have been found to persist for several years after the disappearance of all clinical evidence of rheumatic activity.

*Half Life of Hydrocortisone in Patients with Rheumatic Fever*

The low levels of 17-OHCS in patients with rheumatic fever might be attributable to an increased rate of their utilization by tissue if, as has been postulated, such an increased rate occurs in patients with rheumatic fever. TABLE 2 presents preliminary data concerning the rate of disappearance of intravenously administered hydrocortisone\* in these patients. In this table, the half life of hydrocortisone in untreated rheumatic fever patients is compared with that in normal children. In the rheumatic fever patients, the values range from 98 to 199 minutes with a mean of 136 minutes, whereas in the normal children the values range from 60 to 98 minutes with a mean of 83 minutes. It appears from these data, that the rate of disappearance of free hydrocortisone from the circulation in patients with rheumatic fever is slower—not faster—than normal.

\* Cortef® kindly furnished by Doctor C. J. O'Donovan of the Upjohn Laboratories, Kalamazoo, Mich.



*Blood ACTH in Patients with Rheumatic Fever*

It previously has been demonstrated that adrenalectomized animals<sup>14</sup> and patients with Addison's disease<sup>15</sup> have elevated circulating concentrations of ACTH, presumably reflecting attempts of their homeostatic mechanisms to compensate for their inadequate adrenal cortical function. The characteristic hormone pattern observed in such subjects, low 17-OHCS concentrations despite elevated ACTH concentrations, may be considered as the prototype of the pattern to be expected in subjects with adrenal insufficiency.

The demonstration of low plasma 17-OHCS concentrations in patients in a given clinical category provides no information as to whether the adrenal cortex is failing to respond adequately to stimulation, or whether the stimulation is inadequate because of failure of release of ACTH by the pituitary. Therefore, for more complete evaluation of the pituitary-adrenal functional status in these patients, it is desirable to measure the concentrations of endogenous ACTH in their blood.

A satisfactory method for measurement of endogenous ACTH levels in human blood has become possible recently with the introduction by Sydnor and Sayers<sup>16</sup> of a modification of the original adrenal ascorbic acid depletion technique of Sayers, Sayers, and Woodbury.<sup>17</sup> Although this procedure is exacting and requires 120 to 150 ml. blood, its use has made possible the direct study of pituitary function and the relation of this function to adrenal cortical secretion.

TABLE 3 shows the blood ACTH concentrations, as determined by this technique, in untreated rheumatic fever patients during various stages of the disease. In this table, in order to permit direct comparison of data obtained on different assay days, adrenal ascorbic acid depletion values observed in the assay animals have been expressed in terms of ACTH concentration (milliunits/liter) in the blood sample assayed. Details of the method of calculation involved have been presented elsewhere.<sup>18</sup> Essentially, it consists of employing, as the standard curve for the day, the log dose (ACTH)-response (adrenal ascorbic acid depletion) curve obtained with different doses of standard ACTH on that day and graphically estimating the ACTH concentration in the unknown sample from this curve and the observed adrenal ascorbic acid depletion values. Regardless of the ACTH concentration which might otherwise have

TABLE 3  
BLOOD ACTH CONCENTRATIONS IN PATIENTS WITH RHEUMATIC FEVER

Stage	No. determinations	Blood ACTH	
		No. elevated	Range (mU/L)
"Early acute" R. F.....	5	0	0
"Transitional phase" R. F.....	6	5	0-25.0
"Well-established active" R. F.....	17	15	0-87.8
Inactive R. F.....	17	15	0-17.4
Chorea.....	7	3	0- 6.0
Normal children.....	9	0	0

been ascribed to an unknown blood sample on the basis of this method of calculation, if the mean adrenal ascorbic acid depletion evoked in the assay animals was less than 15 mg./100 gm. adrenal, this response was considered to be within the range of responses which might be produced by saline, and to indicate no detectable ACTH. Although this method can be expected to yield, at best, only rough approximations of the actual blood ACTH concentrations, it is capable of revealing gross elevations of these concentrations.

As shown in TABLE 3, the sensitivity of this technique is not adequate to detect the low concentrations of endogenous ACTH in the blood of normal children. The values obtained in this group consistently are zero. Likewise, during the "early acute" phase of rheumatic fever, none of the patients had a detectable concentration of circulating ACTH. After the first week of illness, however, blood ACTH levels were elevated almost uniformly. Likewise, elevated ACTH levels were observed in patients with inactive rheumatic fever and chorea, such elevations having been observed as late as two years following an attack of acute rheumatic fever.

#### *Adrenal Insufficiency in Patients with Rheumatic Fever*

Thus, in rheumatic fever patients, except during the "early acute" phase of the disease, plasma 17-OHCS concentrations are lower than normal despite elevated concentrations of ACTH. Since the rate of disappearance of these steroids from the circulation is not increased, these data are interpreted as indicating that the patient with rheumatic fever has adrenal insufficiency.

That this adrenal insufficiency is only *relative* rather than *absolute* is indicated not only by the elevations of plasma steroid concentrations which occur during the "early acute" phase of both first and recurrent attacks of rheumatic fever, but also by the adequate response of plasma steroids to a large test dose of exogenous ACTH. TABLE 4 shows the increase in plasma steroid concentration which results 2 hours after the intramuscular injection of 25 I.U. ACTH. The mean increase in 35 rheumatic fever patients (22.7  $\mu$ g. per cent) did not differ statistically from that in 40 control children (17.8  $\mu$ g. per cent).

The data presented indicate the existence of relative adrenal insufficiency in patients with rheumatic fever both during and for years following an acute attack of the disease. They give no indication whether the relative adrenal insufficiency antedates the rheumatic fever or occurs as a result of the disease.

TABLE 4  
PLASMA 17-HYDROXYCORTICOSTEROID RESPONSE TO ACTH STIMULATION IN PATIENTS WITH RHEUMATIC FEVER

Group	Plasma 17-hydroxycorticosteroid concentration		
	No. tests	Mean increase* ( $\mu$ g %)	S.E.M.
Control .....	40	17.8	$\pm 1.76$
Rheumatic fever .....	35	22.7	$\pm 4.19$

\* 2 hours after the intramuscular injection of 25 I.U. lyophilized ACTH.

At the present time, no data are available to differentiate between these two possibilities.

The etiologic role of the Group A beta-hemolytic streptococcus in rheumatic fever is established.<sup>19</sup> The interpretation of the data presented here as indicating the existence of relative adrenal insufficiency in patients with this disease is not to be construed as implying that the symptomatology of the disease is attributable in a direct way to the adrenal insufficiency, or as implying any disparagement of the role of the streptococcus in rheumatic fever.

### *Hormone Therapy in Rheumatic Fever*

The demonstration of adrenal insufficiency in patients with rheumatic fever provides rationale on a physiological as well as a pharmacological basis for therapy with adrenal steroids in this disease. This rationale applies, likewise, to therapy with ACTH, since these patients have relative rather than absolute adrenal insufficiency and respond to ACTH administration with adequate elevations of steroid concentrations.

The remainder of this presentation will be devoted to a discussion of the results of hormone therapy. Since we do not have sufficient experience with hydrocortisone therapy to justify its inclusion, the data to be presented will be concerned only with the results of therapy with cortisone\* or ACTH†. These data have been presented elsewhere in more detail.<sup>20</sup>

Since adrenal insufficiency is an integral part of rheumatic fever, hormone therapy of this disease may be considered in part, at least, as replacement therapy and, as such, it must be highly individualized. In our clinic, individualization of therapy is accomplished by adjusting the size of the dose to the size of the patient and by adjusting the duration of therapy according to the response of the patient. The minimum initial dose employed is one International Unit of ACTH or three mg. cortisone per pound of body weight per day. Therapy with the full initial dose is continued until the following criteria have been met: (1) no clinical evidence of activity remains; (2) the sedimentation rate has been normal for at least one week, and (3) the serum mucoprotein level has decreased at least to six mg. per cent.<sup>21</sup> When these criteria have been met, the dose is very gradually reduced in small steps at two- to three-day intervals. This individualized therapeutic regimen consistently has evoked a prompt subsidence of symptomatology in patients with acute rheumatic fever. In no case has it been necessary to discontinue therapy because of side effects. Clinical rebound has not been observed in any patient treated in strict conformity with the criteria mentioned.

To be of significant value, however, hormone therapy must accomplish more than a mere shortening of the acute symptomatology of the disease. Of much greater import is the influence of therapy on the residual cardiac status of the patient. In an attempt to obtain an objective evaluation of the latter, a comparison has been made of the incidence of residual cardiac murmurs following different therapeutic regimens in patients who were known to have no murmur before the onset of the attack of rheumatic fever. These data are shown in

\* Cortone® supplied through the courtesy of Merck and Co. Inc., Rahway, N. J.

† ACTHAR® and ACTHARGEL® supplied through the courtesy of Armour Laboratories, Chicago, Ill

TABLE 5  
RESIDUAL CARDIAC MURMURS\* FOLLOWING THERAPY OF RHEUMATIC FEVER

Time of exam.	Hormone R <sub>x</sub>		Nonhormone R <sub>x</sub>	
	No. pts. examined	Per cent with murmurs	No. pts. examined	Per cent with murmurs
Discharge	46	52	34	74
3 mo.	35	40	31	77
6 mo.	34	26	27	67
1 yr.	31	16	26	81
2 yr.	25	8	20	75
3 yr.	17	6	17	82

\* Includes all detectable murmurs.

TABLE 5. Eighty patients could be included in this group. Of these, 46 were treated with either ACTH or cortisone, 21 with salicylates, and 13 with bed rest alone. Since no significant difference was found with regard to residual cardiac murmurs either between the two hormone-treated groups or between the salicylate and bed-rest groups in this respect, the results in all hormone-treated patients are compared with those in all patients not treated with hormones. In TABLE 5, the percentage of patients who had detectable residual cardiac murmurs is presented for various follow-up periods. At the time of institution of therapy, all hormone-treated patients, and all but one of those not treated with hormones had murmurs. At the time of discharge from the hospital, 52 per cent of the hormone-treated patients and 74 per cent of those not treated with hormones had residual cardiac murmurs. Thereafter, in the nonhormone treated group, there was no significant change with time in the incidence of residual murmurs whereas, in the hormone group, the incidence of residual murmurs decreased with each successive examination until, at one year, only 5 of 31 patients (16 per cent) had any residual murmur and, in the groups of patients followed for 2 and 3 years, only 8 per cent and 6 per cent respectively, had murmurs. The figure of 6 per cent at 3 years represents 1 of 17 patients. Analysis shows that the difference between the two groups with regard to cardiac residua cannot be attributed to patients lost to follow-up.<sup>20</sup>

TABLE 6 shows the number of patients who developed new cardiac diastolic murmurs or systolic murmurs of grade 2 or greater intensity at varying intervals following discharge from the hospital. The figures refer to the number of patients who developed new murmurs not audible in the immediately preceding follow-up period. No diastolic murmurs or systolic murmurs of grade 2 or greater intensity had their first appearance following discharge from the hospital among the hormone-treated patients. In contrast, among the patients not treated with hormones new diastolic murmurs appeared in six and new systolic murmurs of grade 2 or greater intensity appeared in seven patients. None of these new murmurs was observed to disappear at successive examinations.

Although these observations concerning the influence of hormone therapy in rheumatic fever must be extended before a final, definitive evaluation can be



TABLE 6  
APPEARANCE OF NEW MURMURS DURING THE FOLLOW-UP PERIOD

		Interval since discharge					Total no. new murmurs
		3 mo.	6 mo.	1 yr.	2 yr.	3 yr.	
Hormone treated	Diastolic.....	0	0	0	0	0	0
	Systolic*.....	0	0	0	0	0	0
Not treated with hormones	Diastolic.....	0	2	1	2	1	6
	Systolic*.....	2	0	2	2	1	7

\* Grade 2 or greater intensity.

achieved, the results cited indicate that, in this series of patients, hormone therapy was more efficacious than other forms of therapy employed. Not only did the hormones produce a rapid subsidence of the exudative manifestations of rheumatic activity without "clinical rebound" after cessation of therapy but, also, they appear to have exerted a definite beneficial effect on the residual cardiac status of the patients. As indicated previously, a smaller percentage of the hormone-treated patients had any detectable residual murmurs at various follow-up intervals. In addition, the murmurs which were observed in the hormone group were less serious than those in the nonhormone group. There were no patients in the hormone group with residual cardiac enlargement or electrocardiographic abnormalities.<sup>20</sup> The striking contrast between the hormone-treated group and the group not treated with hormones with regard to appearance of new, significant murmurs at different intervals during the follow-up period suggests that hormone therapy may have terminated rheumatic activity in these patients. These evidences of the beneficial effects of hormone therapy with regard to both acute manifestations of the disease and possible termination of disease activity are in conformity with the thesis proposed that the endocrine pattern observed in patients with rheumatic fever provides rationale for the use of hormone therapy in this disease.

### Summary

Patients with rheumatic fever were found to have a relative adrenal cortical insufficiency manifested by low circulating concentrations of 17-OHCS and high circulating levels of ACTH, with a normal adrenal-steroid response to sufficient exogenous ACTH stimulation. These findings offer a definitive rationale on a physiologic basis for the therapy of rheumatic fever. The results of treatment with ACTH and cortisone as employed in this study are encouraging indeed. The hormone-treated patients in this series demonstrated a prompt subsidence of acute manifestations of rheumatic fever with no clinical evidence of posttherapy reactivation. Most important, however, in contrast to the patients not treated with hormones, the hormone-treated patients only rarely were found to have residual cardiac murmurs after follow-up periods of up to three years. Moreover, the development of new murmurs following discharge from the hospital was rare among the hormone-treated patients but

comparatively common among the patients not treated with hormones. The data presented suggest that these agents actually may be capable of terminating the rheumatic process.

### *Acknowledgments*

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### *References*

1. MASSELL, B. F. 1954. ACTH and cortisone therapy of rheumatic fever and rheumatic carditis. *New Engl. J. Med.* **251**: 183, 221, 263.
2. SAYERS, G. 1950. The adrenal cortex and homeostasis. *Physiol. Revs.* **30**: 241.
3. THORN, G. W., T. B. BAYLES, B. F. MASSELL, P. H. FORSHAM, S. R. HILL, JR., E. SMITH, III & J. S. WARREN. 1949. Studies on the relation of pituitary-adrenal function to rheumatic disease. *New Engl. J. Med.* **241**: 529.
4. DAVISON, R. A., P. KOETS & W. C. KUZELL. 1947. Excretion of 17-ketosteroids in ankylosing spondylarthritis and in rheumatoid arthritis, preliminary report. *J. Clin. Endocrinol.* **7**: 201.
5. DESMARAIS, M. H. L. 1949. The neutral 17-ketosteroids in rheumatoid arthritis and spondylitis. *Ann. Rheumatic Diseases.* **8**: 296.
6. HENCH, P. S., E. C. KENDALL, C. H. SLOCUMB & H. F. POLLEY. 1950. Effects of cortisone acetate and pituitary ACTH on rheumatoid arthritis, rheumatic fever and certain other conditions; a study in clinical physiology. *Arch. Internal Med.* **85**: 545.
7. FREUND, H. A., D. H. BASINSKI & R. B. SCOTT. 1950. 17-Ketosteroid excretion in rheumatoid arthritis. *J. Michigan State Med. Soc.* **49**: 1076.
8. HOWARD, R. P., E. H. VENNING & G. H. FISK. 1950. Studies of adrenocortical and hypophyseal function and the effects thereon of testosterone and pregnenolone therapy. *Can. Med. Asso. J.* **63**: 340.
9. STAUB, P. L., J. W. MENTHE, S. S. NELSON & H. COHN. 1949. Excretion of 11-oxy-corticosteroids in paraplegic and rheumatoid arthritis patients. *J. Clin. Invest.* **29**: 349.
10. THORN, G. W. & T. B. BAYLES. 1949. Current therapeutics; XXII. Pituitary adrenal function and rheumatic disease. *Practitioner.* **163**: 365.
11. NELSON, D. H., L. T. SAMUELS, D. G. WILLARDSON & F. H. TYLER. 1951. The levels of 17-hydroxycorticosteroids in peripheral blood of human subjects. *J. Clin. Endocrinol.* **11**: 1021.
12. NELSON, D. H. & L. T. SAMUELS. 1952. A method for the determination of 17-hydroxycorticosteroids in blood: 17-hydroxycorticosterone in the peripheral blood. *J. Clin. Endocrinol. & Metab.* **12**: 519.
13. KELLEY, V. C., R. S. ELY, A. K. DONE & L. E. AINGER. 1955. Studies of 17-hydroxycorticosteroids. VI. Circulating concentrations in patients with rheumatic fever. *Am. J. Med.* **18**: 20.
14. GEMZELL, C. A., D. C. VAN DYKE, C. A. TOBIAS & H. M. EVANS. 1951. Increase in the formation and secretion of ACTH following adrenalectomy. *Endocrinology.* **49**: 325.
15. SYDNOR, K. L., G. SAYERS, H. BROWN & F. H. TYLER. 1953. Preliminary studies on blood ACTH in man. *J. Clin. Endocrinol. & Metab.* **13**: 891.
16. SYDNOR, K. L. & G. SAYERS. 1952. A technic for determination of adrenocorticotrophin in blood. *Proc. Soc. Exptl. Biol. Med.* **79**: 432.
17. SAYERS, M. A., G. SAYERS & L. A. WOODBURY. 1948. The assay of adrenocorticotrophic hormone by the adrenal ascorbic acid-depletion method. *Endocrinology.* **42**: 379.
18. BRILL, A. B., R. S. ELY, A. K. DONE, L. E. AINGER & V. C. KELLEY. Blood adrenocorticotrophin (ACTH) in children with rheumatic fever. Submitted for publication.
19. SCHWENTKER, F. F. 1952. The epidemiology of rheumatic fever. *In Rheumatic Fever.* : 17. L. Thomas, Ed. Univ. Minnesota Press. Minneapolis, Minn.
20. DONE, A. K., R. S. ELY, L. E. AINGER, J. R. SEELY & V. C. KELLEY. 1955. Therapy of acute rheumatic fever. *Pediatrics.* In press.
21. KELLEY, V. C., F. H. ADAMS & R. A. GOOD. 1953. Serum mucoproteins in patients with rheumatic fever. *Pediatrics.* **12**: 607.

# HYDROCORTISONE IN THE THERAPY OF ASTHMA\*

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## *Introduction*

Preceding papers have indicated that much is known about the pharmacology of hydrocortisone, but they have clearly showed that much remains to be clarified. Unfortunately the disease, asthma, remains a mystery. Combining two partially known factors with the hope of producing a known factor is not rational. The history of asthmatic therapy is studded with preparations and procedures which long ago have been discarded. Only careful study of the patient with asthma before, during, and after withdrawal of therapy will lead to even incomplete answers. With one steroid compound following another with considerable speed, it is difficult to test each succeeding agent with any degree of care. Studies here reported, conducted at the Massachusetts General Hospital (M.G.H.), are only a little over a year in duration and must be weighed accordingly.

## *Methods*

All asthma may be placed in one of two broad groups: first, asthma in which a specific or number of specific antigen-antibody reactions can be demonstrated; and, second, asthma in which no antigen-antibody reaction can be found. Each patient in these studies was known to the clinic for a reasonable length of time. Every attempt to establish an etiological diagnosis was made. When antigen-antibody reactions were demonstrated, patients were removed from the responsible antigens or hyposensitization was attempted whenever removal from antigenic substances was not practical. Seriously ill patients entered the hospital, where good nursing care was provided. Established forms of anti-asthmatic therapy always received adequate trial. Only after the failure of routine conservative measures was hydrocortisone therapy undertaken.

In every patient, the following studies were done before beginning treatment with hydrocortisone: complete history and physical examination, roentgenogram of chest, urinalysis, and complete blood count. When maintenance therapy was a possibility, additional studies included: daily circulating eosinophils; fasting blood glucose; nonprotein nitrogen; serum electrolytes (chloride, sodium, potassium); serum carbon dioxide; serum calcium; serum alkaline phosphatase and phosphorus; three 24-hour urines for calcium; and roentgenograms of the vertebral column. All patients were placed on a moderately low sodium diet (not over 1 gram daily).

Hydrocortisone dosage was determined by trial and error. Initially, enough hydrocortisone was given to suppress symptoms completely. The daily dose was then gradually reduced until the patient was off hydrocortisone or until symptoms began to reappear. In the latter case, the proper daily maintenance

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TABLE 1  
GENERAL PRINCIPLES

- 
- (1) Hydrocortisone does not supplant investigative procedures.
  - (2) Older preparations such as epinephrine precede hydrocortisone.
  - (3) Patient supervision and cooperation are essential.
  - (4) Hydrocortisone is not a "magic drug."
    - (a) Its action is slow. If it is to be used, do not wait until all is lost.
    - (b) Use it in conjunction with other measures.
- 

dose was usually just above this level. Each patient was seen at regular intervals of one or two weeks, and 24-hour medical service was available. Laboratory studies were repeated at chosen intervals.

### *General Considerations and Contraindications*

Steroid therapy in asthma is relatively new, but certain general principles have already been established. TABLE 1 lists some of these principles. In asthma, the most important part of therapy is to establish an etiological diagnosis. Failure to discover specific antigens dooms the patient to symptomatic therapy. If ephedrine, epinephrine, iodides, or aminophylline controls symptoms adequately, hydrocortisone should not be used. Patient supervision and cooperation are essential for success. The patient should be acquainted with possible side effects. The physician must be available for any untoward reactions. Patients should not be started on hydrocortisone only to have the physician proceed to Florida for a month unless another physician with the patient's record is willing and able to take over. All patients must report the slightest variation from normal at once to the responsible physician. Last but not least, hydrocortisone is not a "magic drug." Do not wait until the patient is almost dead before starting hydrocortisone, which does not act rapidly. If the waiting period is too long, some patients may not live long enough to be benefited. While waiting for the full effect of hydrocortisone, epinephrine and aminophylline may give some relief.

Before using hydrocortisone, certain possible contraindications should be considered. TABLE 2 lists a group of these, but the final decision rests with the physician who must decide, as always, whether the risks of therapy are justified because of the seriousness of the disease. Several workers<sup>1, 2, 3</sup> showed that ACTH and steroid therapy can accelerate tuberculosis. Ebert and Barclay<sup>4</sup> demonstrated that cortisone improved the circulation of small blood vessels in the area of a tuberculous process. Recently Sors and Trocmé<sup>5</sup> and Cochran<sup>6</sup> have used ACTH and cortisone in conjunction with the antibi-

TABLE 2  
POSSIBLE CONTRAINDICATIONS TO HYDROCORTISONE

- 
- (1) Tuberculosis.
  - (2) Acute infections.
  - (3) Gastrointestinal diseases. (Ulcer, Ulcerative colitis)
  - (4) Psychosis.
  - (5) Osteoporosis.
  - (6) Thromboembolic phenomena.
-



otics in the treatment of tuberculosis with reported excellent results. Browne *et al.*<sup>7</sup> have been able to give patients with coexisting Addison's disease and active pulmonary tuberculosis cortisone in relatively small doses (12.5 to 37 mg. daily) plus appropriate antibiotics without deleterious effects. As pointed out by others,<sup>8, 9, 10</sup> gastrointestinal complications do occur during steroid therapy, and those with gastrointestinal disease may well be poor risks. A diabetic-like state has been reported to be induced by ACTH and steroid therapy, but it disappears once therapy is discontinued. Psychoses have been reported by several<sup>11, 12, 13</sup> during ACTH and cortisone but, again, withdrawal returns the patient to normal within varying periods. Osteoporosis with vertebral fractures has been reported in patients receiving maintenance steroid therapy.<sup>14, 15, 16</sup>

### *Use in "Status Asthmaticus"*

Both patients with asthma based on antigen-antibody reactions and those with asthma of undetermined etiology do develop "status asthmaticus," which may be described as asthma which is severe, life-threatening, and unresponsive to the usual therapy. Hydrocortisone can be effective in these patients. Frequently the gastrointestinal tract of such patients is upset because of symptoms plus preceding therapy. The intravenous form of hydrocortisone, therefore, is the preparation of choice. Two-hundred mg. of hydrocortisone in 1000 ml. of 5 per cent glucose in water as a slow intravenous drip over eight hours are usually effective. This treatment is followed by 40 to 50 mg. of oral hydrocortisone every six hours until symptoms of asthma are suppressed. Intravenous therapy may be continued for a longer period if a patient's condition warrants it. Once the symptoms are suppressed, the daily dose is decreased at the rate of 20 mg. daily until the patient is off hydrocortisone. If symptoms return as the dose is decreased, it is often wise to return to a dose just above this level and maintain the patient symptom-free for a period of time. No patient with extrinsic asthma should be maintained on hydrocortisone for long periods. In certain cases of pollen asthma in whom hyposensitization and conventional therapy have failed, maintenance therapy of hydrocortisone, at the lowest level that will suppress symptoms, is justified for that particular pollen season.

### *Maintenance Hydrocortisone*

In those patients with severe, daily asthma which has led to social and economic invalidism, long-term maintenance hydrocortisone may be useful. The initial therapy is similar to that described under "status asthmaticus." Before maintenance therapy is started, it is imperative that the patient be freed completely of symptoms. During the following week, the daily dose is gradually reduced to about 100 mg. The daily dose is then further reduced by 20 mg. every week or two until symptoms return. The maintenance level is usually a few milligrams more than that of the level where symptoms begin to return. At least several efforts to lower this maintenance level should be attempted. TABLE 3 lists several phenomena of maintenance hydrocortisone which have been observed.

TABLE 3  
HYDROCORTISONE MAINTENANCE

- (1) The optimal daily dose is the lowest which provides freedom of symptoms.
- (2) Trial and error is the method of finding maintenance level.
- (3) Maintenance dose does not depend upon:
  - (a) Body mass.
  - (b) Severity of asthma.
  - (c) Age of patient.
  - (d) Duration of asthma.
- (4) Infections and stress increase maintenance level.
- (5) One dose every 12 hours is best.
- (6) If asthma returns, failure to raise dose may lead to severe asthma.

TABLE 4  
MAINTENANCE HYDROCORTISONE

Name	Sex	Age	Initial clearing dose in mg. hydrocortisone	Days to clear	Mean daily dose in mg. hydrocortisone
C. E.	M	55	1150	6	42
E. H.	F	59	1100	6	54
G. Mc.	M	57	1000	5	50
M. P.	F	62	1950	10	47
S. W.	F	48	1510	8	38
G. W.	F	74	675	4	55

For the past year, six patients with severe, intractable asthma of unknown etiology have been maintained free of symptoms with daily doses of hydrocortisone. TABLE 4 lists these patients with pertinent data.

### Complications

To date, little difficulty with initial therapy has been encountered. The daily dose of hydrocortisone in maintenance therapy for long periods, however, probably represents hyperadrenocorticism in most instances. All six of these patients show some of the mild manifestations of hyperadrenocorticism, such as an increase of facial hair in females, acne in males, facial rubor, rounding of facial contours, and abnormal distribution of fat in the abdominal area, supra-clavicular fossae, and lower cervical region. All have gained weight.

In studies to date, one patient, not included in TABLE 4, has developed osteoporosis with vertebral fractures. At present she is off hydrocortisone. FIGURE 1 diagrams her problem.

*Case of B. B., female, white, 52 years—M.G.H. No. 243264.*

Asthma first appeared in 1942 and was only of moderate severity at first. It gradually became more persistent and severe. In December 1953, she had to stop work. First admission to M.G.H. was in February 1954. No etiological diagnosis was established, but respiratory infections did seem to play a part. Intensive therapy was only partially effective. Finally, on March 5, 1954, she was admitted for study.

Her past medical history included: removal of right ovary at age 18 (reason

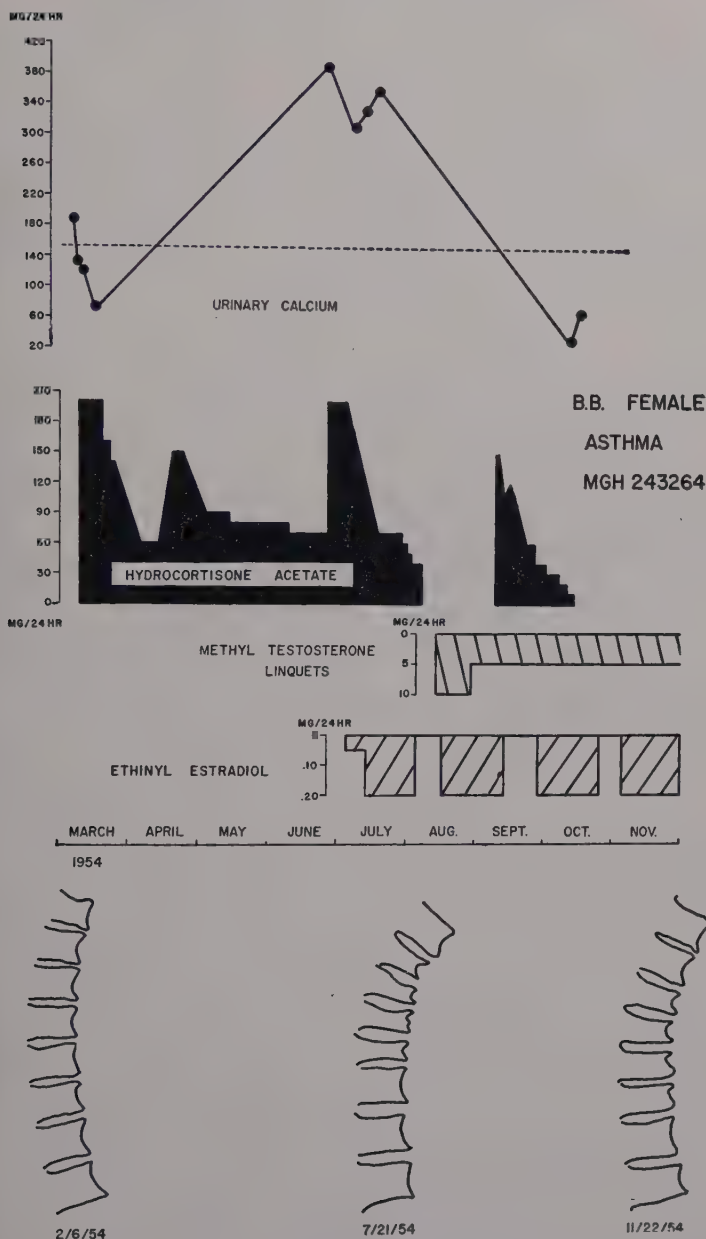


FIGURE 1

not known); removal of left ovary and uterus in 1944 (benign tumor of uterus); and cholecystectomy in 1951.

Because of artificial menopause and because of densities in the apex of the right lung, she was not considered a good candidate for maintenance hydro-

cortisone. On the other hand, her symptoms were not adequately controlled by other methods and she was invalided by her asthma. Moreover, roentgenograms of her vertebrae appeared normal and her 24-hour urine calcium excretions were within normal limits. Hydrocortisone therapy was therefore decided upon as a calculated risk and was started on March 8, 1954. She cleared only after 3940 mg. had been given, over a 2-week period. Her daily maintenance level was about 150 mg., a high figure. By late April 1954 she had back pain and, by July, roentgenograms of the vertebrae showed extensive osteoporosis and several compressed vertebrae. Estrogen and testosterone therapy was started in July 1954 in an effort to prevent further decalcification of her bones. At present she is off hydrocortisone; her asthma is severe again.

### *Summary and Conclusions*

These studies indicate that hydrocortisone is effective in "status asthmaticus" and in patients with severe intractable asthma of unknown etiology. These patients may be maintained symptom-free on daily doses of hydrocortisone for long periods of time if the daily level of hydrocortisone is maintained at an optimal level which is found by "trial and error." Such maintenance hydrocortisone may represent manifestations of Cushing's syndrome. The most serious complication to date has been osteoporosis. Determination of 24-hour urine calcium excretion and periodic vertebral roentgenograms may prove to be guides in predicting which patients are developing clinical osteoporosis. Films of the spine must not be considered infallible, however, as they may appear normal while marked decalcification exists. Only cooperative patients under the constant supervision of interested physicians should receive maintenance hydrocortisone.

### *References*

1. FREEMAN, S., J. FERSHING, C. C. WANG & L. C. SMITH. 1950. Proceedings of the First Clinical ACTH Conference. Blakiston. Philadelphia, Pa.
2. FRED, L., M. H. LEVIN, J. B. RIVO & T. F. BARRETT. 1951. Development of active pulmonary tuberculosis during ACTH and cortisone therapy. *J. Am. Med. Assoc.* **147**: 242.
3. KING, E. Q., J. B. JOHNSON, G. S. BATTEN, & W. L. HENRY. 1951. Tuberculosis following cortisone therapy. *J. Am. Med. Assoc.* **147**: 238.
4. EBERT, R. H. & W. R. BARCLAY. 1952. Changes in connective tissue reaction induced by cortisone. *Ann. Internal Med.* **37**: 506.
5. SORS & Y. TROCMÉ. 1954. Le traitement des pleurésies serofibrineuses tuberculeuses par l'ACTH. *Presse méd.* **62**: 268.
6. COCHRAN, J. B. 1954. Cortisone in treatment of pulmonary tuberculosis. *Edinburgh Med. J.* **61**: 238.
7. BROWNE, J. S. L., M. ARONOVITCH, J. C. BECK, W. LEITH & J. W. MEALSINS. 1954. The treatment of coexisting Addison's disease and active pulmonary tuberculosis. *Am. J. Med. Sci.* **228**: 411.
8. TRUELOVE, S. C. & L. J. WITTS. 1954. Cortisone in ulcerative colitis. *Brit. Med. J.* **2**: 375.
9. SAUER, W. G., W. H. DEARING & E. E. WOLLAEGER. 1953. Serious untoward gastrointestinal manifestations possibly related to administration of cortisone and corticotropin. *Proc. Staff Meetings Mayo Clinic.* **28**: 641.
10. BOSIEN, W. R. & M. D. TYSON. 1953. Spontaneous perforation of a benign gastric ulcer into the transverse colon: report of a case. *Gastroenterology.* **24**: 113.
11. BORMAN, M. C. & H. C. SCHMALLENBERG. 1951. Suicide following cortisone treatment. *J. Am. Med. Assoc.* **146**: 337.



12. CLARK, L. D., W. BAUER & S. COBB. 1952. Preliminary observations on mental disturbances occurring in patients under therapy with cortisone and ACTH. *New Engl. J. Med.* **246**: 205.
13. CLARK, W. S., H. O. TONNING, J. P. KULKA & W. BAUER. 1953. Observations on the use of cortisone and ACTH in rheumatoid arthritis. *New Engl. J. Med.* **249**: 635.
14. DEMARTINI, F., A. W. GROKOST & C. RAGAN. 1952. Pathological fractures in patients with rheumatoid arthritis treated with cortisone. *J. Am. Med. Assoc.* **149**: 750.
15. IRWIN, J. W., P. H. HENNEMAN, D. M. K. WANG & W. S. BURRAGE. 1954. Maintenance cortisone in intractable asthma: preliminary observations of undesirable cortisone effects. *J. Allergy.* **25**: 201.
16. CURTIS, P. A., W. S. CLARK & C. H. HERNDON. 1954. Vertebral fractures resulting from prolonged cortisone and corticotropin therapy. *J. Am. Med. Assoc.* **156**: 467.

### *Discussion of the Paper*

DOCTOR RAGAN: I have two questions which I should like to ask Doctor Burrage. (1) Is it necessary, or has it been found advisable, to administer ACTH as hydrocortisone dosage is decreased? (2) Have you found any evidence of adrenal insufficiency when you have switched to hydrocortisone in any patients?

DOCTOR BURRAGE: The answer to the first question is that we really do not know, and that we have not tried. The answer to the second question is that we have had no trouble in that respect. We have had trouble when we have taken patients off cortisone in the past. Each of the members of our larger series of cortisone patients has been taken off, but we have not had any difficulty when we switched.

QUESTION: I should like to ask one general question. During the past year we had 60 compression fractures in postmenopausal women who had been on corticoids. Have you seen compression fractures in other than postmenopausal women?

DOCTOR RAGAN: Yes, I have. I have seen them in rheumatoid males and premenopausal women.

## THE USE OF HYDROCORTISONE IN ULCERATIVE COLITIS: PRELIMINARY OBSERVATIONS\*

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### *Introduction*

Ulcerative colitis is an acute and chronic inflammatory and ulcerative disease of the colon and rectum, occurring chiefly in young people.<sup>1</sup> Its cause is not known. The principal symptoms are bloody diarrhea, cramping abdominal pain, malaise, fever, and weight loss. The course is characterized by unpredictable remissions and exacerbations and by numerous complications, including infections, nutritional deficiencies, polypoid hyperplasia, hemorrhage, stenosis, obstruction, and perforation of the bowel. There is no specific treatment. Therapy is symptomatic, adapted to the individual case and prolonged. The results occasionally are excellent. Frequently, however, the response is unsatisfactory or erratic. Colectomy and ileostomy are necessary in approximately 10 or 15 per cent of patients because of serious complications or lack of improvement during medical management.

Previous studies by us<sup>2, 3</sup> and by others have demonstrated that corticotropin and cortisone, though not curative, may be useful therapeutic adjuncts in ulcerative colitis. The beneficial effects of corticotropin usually are rapid and, at times, dramatic. The principal disadvantages of ACTH are the need for intramuscular or intravenous injection, the frequency of side effects and, occasionally, the diminishing response to prolonged administration.<sup>4</sup> Cortisone has the advantages of oral administration and fewer complications but, in the amounts prescribed, is considerably less beneficial. The development of hydrocortisone suggested the possibility of steroid treatment more effective than cortisone, approximating the potency of corticotropin, yet administered easily and safely by mouth. The present paper summarizes observations since May 1953 on the course of 40 patients with ulcerative colitis treated with hydrocortisone.

*Patients.* The series includes 23 females and 17 males. The age range usually was 20 to 40. Six were children less than 12 years of age. The colitis was classified as mild in two cases, moderately severe in 19, and severe in 19. The duration of symptoms among individual patients was 6 weeks to 15 years; less than 1 year in 3; 1 to 5 years in 16; 5 to 10 years in 11; and longer than 10 years in 10 patients. Frequent or prolonged hospitalization had been necessary in most instances. Colectomy and ileostomy had been recommended elsewhere in at least 13 of the group. X rays of the large bowel were obtained in 39 cases; the entire colon was involved in 18; varying portions of the bowel were affected in 18; the colon appeared normal in three (TABLE 1). Moderate or severe active colitis was observed at proctoscopy in 28 patients. There was roentgen evidence of the disease proximal to the rectosigmoid in the 12 individ-

\* This study was supported in part by the Wallach Fund for Research in Gastroenterologic Diseases, University of Chicago, Chicago, Ill.

Hydrocortisone was supplied in generous quantities by the Upjohn Company, Kalamazoo, Mich.

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TABLE 1  
ROENTGEN EXTENT OF ULCERATIVE COLITIS

	No. patients
Normal*	3
Left colon	9
Left colon and transverse	6
Right colon	3
Entire colon	18
Undetermined	1
Total.....	40

\* Active colitis at proctoscopy.

TABLE 2  
PROCTOSCOPIC SEVERITY OF ULCERATIVE COLITIS

	No. patients
Normal*	4
Mild*	8
Moderate	10
Severe	17
Undetermined	1
Total.....	40

\* Roentgen involvement proximal to rectosigmoid.

uals with normal or minimal changes (TABLE 2). Complications were present in 31 patients. They included anemia, 16; polyposis or pseudopolyposis, 6; arthritis, 4; skin lesions, 3; anal fistulae, 3; hepatic disease, 2; uveitis, 1; and, in one patient, both uveitis and iridocyclitis. In addition, there were two individuals with peptic ulcer and single patients with diabetes mellitus, hypertensive cardiovascular disease, and healed, previously extensive pulmonary and osseous tuberculosis. Emotional problems were recognized in 24 patients; 9 of this group had received psychiatric attention. The presence of psychogenic disturbances in many, if not all of the remaining cases seems likely.

*Previous laboratory studies.* The feces consistently were negative for pathogenic bacteria and parasites. X rays of the chest were normal. In small groups of cases, the following procedures also were negative or normal: skin tests for lymphopathia venereum, histoplasmosis, and coccidioidomycosis, serum agglutinins against enteric pathogens, oral glucose tolerance test, serum calcium and phosphorus, basal metabolism, gastric secretion, and tests for "L. E. cells."

### *Method of Study*

The procedure has been described previously.<sup>2, 3</sup> All patients had been under careful supervision, and the course of their illness was known. The response to medical treatment had been poor or the disease had recurred after initial improvement. With one exception, therapy was initiated in the hospi-

TABLE 3  
QUANTITY OF HYDROCORTISONE  
(Totals for Individual Patients)

Quantity	No. patients
gm.	
<5	7
5-10	14
10-15	13
15-20	2
>20	4
Total.....	40

Range: 1.5 to 23.0 gm.

tal. Following hospitalization, patients returned at regular intervals in the out-patient department. An additional period of observation during medical management without steroids preceded the administration of hydrocortisone. The control aspects of steroid therapy in ulcerative colitis have been considered previously.<sup>2, 3</sup> Treatment included a bland, nutritious diet, sedatives, antispasmodics, and vitamins. Sulfonamides, usually sulfaguanidine 8.0 gm. daily, were administered routinely. Azulfidine, 4.0 gm., and gantrisin, 2.0 gm. daily, were prescribed occasionally. Penicillin and streptomycin were given in a few cases. The intake of salt was not restricted except to control obvious retention of fluid. Mercurial diuretics also were effective for this purpose. Potassium salts were not prescribed. Twenty-one patients previously had received at least one course of therapy with ACTH. Eight of this group at another time also had taken cortisone. Cortisone only had been administered in two cases.

*Administration of hydrocortisone.* Hydrocortisone was prescribed initially in quantities of 200 mg. by mouth daily in four divided doses. The amount was increased to 300 mg. after one or two weeks, if the clinical response was inadequate. With subsidence of symptoms, the dose was reduced by decrements of 10 or 25 per cent, at intervals of one or two weeks in the hospital and, later, at lower dosage levels, every three to six weeks (TABLES 3 and 4). The amounts of hydrocortisone required to control ulcerative colitis in the six chil-

TABLE 4  
DURATION HYDROCORTISONE THERAPY  
(Totals for Individual Patients)

Duration	No. patients
(months)	
<1	4
1-3	19
4-8	11
9-12	6
Total.....	40

22 patients continue treatment.



TABLE 5  
INITIALLY EFFECTIVE DAILY DOSES OF HYDROCORTISONE (MGM.)

	Adults (34)	Children (6)
Range.....	80-350	100-300
Average.....	200	150

dren approximated adult doses quantitatively but were proportionately larger in relation to body surface area (TABLE 5). Three patients were given hydrocortisone as ACTH was withdrawn. The initial dose was 20 to 40 mg., increasing to 100 mg. daily when corticotropin was discontinued entirely. In these cases, hydrocortisone was prescribed to maintain the improvement established by corticotropin. Single doses of corticotropin gel were injected intermittently in two patients for the purpose of maintaining a functionally responsive adrenal cortex.

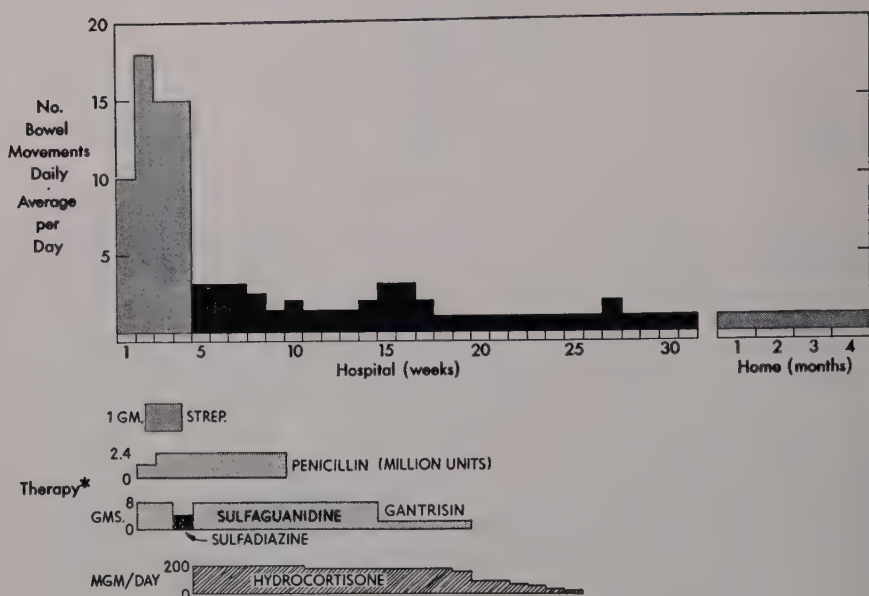
*Observations during treatment.* In addition to careful clinical observation, proctoscopy was repeated frequently. Laboratory studies were limited to the usual blood counts, examination of the feces for occult blood, determination of glucose in the blood and urine and frequent measurements of the serum electrolytes.

### Results

*Immediate effects.* The initial response to hydrocortisone was estimated as good in 18 patients, moderately favorable in 15, and slight or nil in 7 cases (TABLE 6). The course was characterized by a significant reduction in the number of bowel movements, improved consistency of the feces, and disappearance of abdominal pain and rectal bleeding. The decrease in diarrhea in one patient is illustrated graphically in FIGURE 1. Although the improvement occasionally was prompt, the response, in contrast to ACTH, usually was gradual, developing over periods of several days to one or two weeks. The increased appetite and sense of well-being during treatment were less pronounced than during the use of corticotropin. The elevated temperature and tachycardia in six patients subsided within several days after the onset of treatment with compound F, less promptly than after ACTH. The proctoscopic appearance of the bowel improved in most cases, but more slowly than the clinical

TABLE 6  
RESPONSE OF ULCERATIVE COLITIS DURING HYDROCORTISONE THERAPY

Severity of disease	Response			Totals
	Good	Moderate	Slight or nil	
Mild.....	2			2
Moderate.....	8	10	1	19
Severe.....	8	5	6	19
Totals.....	18	15	7	40



\*also Psychotherapy, Sedatives, Antispasmodics, Diet

FIGURE 1. Favorable response of severe ulcerative colitis during and after hydrocortisone therapy.

response. The excessive bloody fluid in the rectum and the mucosal edema disappeared first. The hemorrhagic areas and bleeding subsided more gradually. Reversibility to a completely normal mucosa was not observed during the period of observation.

*Course after hydrocortisone.* Hydrocortisone was discontinued in five patients because of initial lack of improvement. Among 13 patients responding initially in whom therapy has been terminated, symptoms have recurred in 5. Eight patients remain in satisfactory health without compound F. The period of observation, however, is brief, seven months or less. One of this latter group, though continuing in satisfactory health, very recently has experienced a partial return of the diarrhea. Hydrocortisone therapy continues in 22 patients. The response in two thus far has been slight.

*Therapeutic failures.* Twelve patients are classified at present as failures. Seven of this group did not improve initially. In five, the early favorable response was not maintained. With one exception, the colitis in each case was severe. Emotional problems may have contributed to the unsatisfactory responses in at least six instances. This factor, however, is extremely difficult to evaluate. Of the 12 failures, 4 later improved, at least temporarily, during the administration of corticotropin. One woman died during subsequent ACTH therapy, the only fatality in the series. In the absence of an autopsy, the exact cause of death is not known. The other three patients remain in the hospital and may require surgery. Among the remaining eight patients in this category, two are receiving hydrocortisone in the hospital; six continue partially incapacitated in spite of medical treatment without steroids.

*Complications.* There were no serious complications during the administration of hydrocortisone. An obvious Cushing-like appearance developed in 21 cases; it was pronounced in only 4 patients, and therapy was continued in all instances. Retention of fluid and a tendency to Cushing-like facies probably occurred in most patients, but the changes were less apparent. Fluid retention and edema were controlled easily by the restriction of salt and the occasional injection of mercurial diuretics. Mild alkalosis developed in six patients, the serum electrolytes returning to normal gradually when therapy was discontinued or when the quantity of hydrocortisone was reduced. Hypokalemia was not observed. Leukocytosis occurred in 12 patients; the counts usually did not exceed 15,000. One patient with diabetes, well-controlled with 10 units of NPH insulin daily, required 30 units while receiving 100 mg. hydrocortisone daily. The glycosuria was not eliminated entirely. Nevertheless, the diabetes was controlled more easily than during the use of corticotropin. A duodenal ulcer was demonstrated roentgenologically in a young man receiving 300 mg. hydrocortisone daily. The presence of the ulcer before steroid therapy cannot be excluded since the symptoms were very slight and roentgen studies had not been made earlier. The ulcer healed partially after several weeks of antacid treatment, though 200 mg. hydrocortisone were administered daily. There were no additional problems related to the digestive tract. Another man with a known duodenal ulcer received 6.88 gm. of compound F in 87 days without untoward effects. One woman described a peculiar sensation of pressure in the head and the ears; a similar, more intense symptom had accompanied the administration of ACTH one year earlier. The serious complications of severe alkalosis, hypokalemia, hypertension, infection, osteoporosis, hemorrhage, ulceration, and perforation of the bowel, or psychosis, reported occasionally during the use of corticotropin, were not encountered in this series.

*Comparison of hydrocortisone and corticotropin.* Of the 40 patients, 21 previously had received aqueous corticotropin intramuscularly in total amounts of 1000 to 26,000 units, averaging approximately 60 units daily for periods of 16 days to 2 years; five also had taken corticotropin gel. The clinical effects of hydrocortisone appeared superior to those of ACTH in three cases, similar in nine, and inferior in nine patients. The general response of ulcerative colitis, however, was much more striking to ACTH than to hydrocortisone. The gradual improvement observed proctoscopically during the administration of the two compounds was similar.

ACTH produced side effects in all 21 of this group. Hydrocortisone caused obvious side effects in 15. These effects were less severe than had been observed with ACTH in 10 cases and approximately the same in 5 instances. The reactions to hydrocortisone never exceeded those induced by corticotropin. Three patients, while responding to ACTH, developed side effects of such severity (steroid diabetes, psychosis, severe edema) as to necessitate discontinuance of the medication. Hydrocortisone subsequently produced comparable clinical improvement in the same cases but without serious complications. The entire series includes seven patients responding equally to hydrocortisone and to ACTH but with fewer side effects during the administration of compound F. On the other hand, 4 of the 12 failures with hydrocortisone later improved when given corticotropin.

The clinical effects of hydrocortisone could be compared with cortisone in 10 cases. In each instance, compound F appeared more effective. The side effects with hydrocortisone were similar or less pronounced. Similar observations have been reported in rheumatoid arthritis.<sup>5, 6</sup>

### *Comment*

The present study demonstrates that hydrocortisone (compound F) may be a useful adjunct in the treatment of ulcerative colitis when administered orally in sufficient quantities. The favorable results, initially, in 33 of 40 patients, are noteworthy, regardless of subsequent course. The colitis was severe in most instances and had not improved consistently during comprehensive medical management. Though colectomy and ileostomy had been recommended elsewhere in 13 cases, surgery has not been required thus far, and remains under consideration in only three cases. The improvement is not attributable exclusively to hydrocortisone, since therapy included diet, rest, sedation, antispasmodics, sulfonamides, and other supportive measures. The possible psychotherapeutic effects of new, carefully-supervised medication in ulcerative colitis likewise cannot be disregarded entirely but, in the present selected series, this factor appeared to be negligible.

The response to hydrocortisone did not differ demonstrably from the improvement observed during conventional therapy, but it was more frequent in occurrence and more rapid in development. Although the favorable results with hydrocortisone compare arithmetically with the use of corticotropin in a similar series of 40 patients reported in 1951<sup>2</sup> (TABLE 7), ACTH was far more potent in controlling the severe acute manifestations of the disease. The principal advantage of hydrocortisone was the infrequency of important side effects. In the present study, the hormone was administered in quantities up to 23 gm. and for periods up to 353 days without undesirable reactions. The endogenous hydrocortisone produced by the adrenal cortex in response to the dosages of ACTH used in this study probably exceeded the amounts of exogenous hydrocortisone prescribed.<sup>4</sup> Larger quantities of compound F might have produced more striking clinical responses and, at the same time, more pronounced side effects than were observed. Likewise, the disease might have been controlled with smaller doses of corticotropin than were prescribed, thus decreasing the tendency to complications. ACTH remains the compound of

TABLE 7  
COMPARISON OF CLINICAL RESPONSES DURING CORTICOTROPIN AND DURING  
HYDROCORTISONE THERAPY IN ULCERATIVE COLITIS

Severity disease	ACTH (1951)		Compound F (1955)	
	Totals	Satisfactory responses	Totals	Satisfactory response
Mild.....	6	5	2	2
Moderate.....	12	11	19	18
Severe.....	22	18	19	13
Totals.....	40	34	40	33



choice, in our experience, when steroids are indicated in the treatment of severe, active colitis. Hydrocortisone is more useful, perhaps, in the less seriously ill patient and as an adjunct in prolonged treatment, after the acute manifestations have been controlled by corticotropin. This procedure was utilized effectively in three patients. The prolonged use of cortisone in relatively small amounts similarly has been recommended in selected patients with rheumatoid arthritis.<sup>3</sup>

The suppression of adrenocortical function during the long-continued administration of hydrocortisone or cortisone has made desirable intermittent stimulation of the adrenals with ACTH.<sup>8</sup> In two patients, however, single doses of corticotropin gel at weekly or monthly intervals failed to stimulate the adrenal cortex, as measured by the six-hour eosinopenic response. Perhaps the technique requires revision, for the concept seems reasonable physiologically and useful clinically.

The mechanisms of the remissions induced by hydrocortisone, by corticotropin and, indeed, by nonsteroid therapy in ulcerative colitis is not known. Certain theoretical possibilities, including nonspecific alterations in tissue permeability and changes in the hypersensitive state, antigen-antibody relationships and obscure enzyme systems have been mentioned in earlier papers.<sup>2, 3</sup> Various fundamental aspects are discussed elsewhere in this monograph. The development of a technique for producing experimental colitis comparable to the natural disease in man would be extremely valuable for this purpose.

Despite the initially favorable responses, there is no evidence that hydrocortisone eliminates the underlying cause or causes of ulcerative colitis. Thus far, symptoms have recurred in 5 of the 13 patients improving initially and in whom treatment has been discontinued. The period of observation in the 8 cases with clinical remissions is brief. Additional recurrences, therefore, may be anticipated, as noted with corticotropin. On the other hand, 28 of the 40 patients at present are living a relatively comfortable and useful life, with and without compound F. Like corticotropin, hydrocortisone does not cure ulcerative colitis, prevent recurrences, or replace accepted methods of treatment. Nevertheless, when administered under carefully supervised conditions, compound F appears to be a helpful adjunct, initiating and potentiating the process of recovery, permitting the prolonged use of steroids when desirable, and providing a further opportunity for the sustained control of this complex disease. Prolonged observation will be required to evaluate conclusively the ultimate effects of hydrocortisone, corticotropin, and cortisone upon the natural history of ulcerative colitis.

### *Summary*

Since May 1953, 40 patients with chronic ulcerative colitis have been treated with hydrocortisone. Nineteen cases were classified as severe, 19 moderately severe, and 2 as mild. None previously had responded consistently to conventional therapy. Twenty-one patients had received corticotropin previously or were taking the drug immediately preceding hydrocortisone therapy. Cortisone had been prescribed at different times in 10 patients. Hydrocortisone was administered orally as an adjunct to treatment with diet, sulfonamides,

sedatives, antispasmodics, and other supportive measures. The duration of therapy ranged from 23 to 353 days. The total intake of hydrocortisone varied from 1.5 to 23 gm.

The response during treatment was considered good in 18, moderate in 15, and slight or nil in 7 cases. The clinical course was characterized by the disappearance of bloody diarrhea, abdominal pain, and fever, and by a moderately increased appetite and sense of well-being. The proctoscopic appearance of the bowel improved at a slower rate. Treatment continues in 22 patients. Two of this group are not improving satisfactorily. Therapy has been completed in 18 cases, in 5 because of initial lack of improvement. Of the 13 patients in this group responding initially, symptoms have recurred in 5. Eight have maintained their improvement without compound F, but the period of observation is relatively brief. The entire series, therefore, includes 12 therapeutic failures; 5 not responding initially and no longer taking hydrocortisone; 2 not improving but continuing therapy; and 5 in whom symptoms returned when the medication was discontinued.

Comparison between corticotropin and hydrocortisone in 21 patients indicated better results with hydrocortisone in three cases. Corticotropin excelled in nine. The effects were similar in nine patients. The responses of the disease to hydrocortisone was considerably less pronounced than to ACTH. On the other hand, the side effects during effective hydrocortisone therapy, in the doses employed, were less frequent and less severe than those accompanying effective quantities of corticotropin. There were no serious complications during the administration of hydrocortisone. The obvious side effects noted in 21 of the 40 patients were chiefly the Cushing-like appearance and alkalosis.

### *Conclusions*

(1) Hydrocortisone does not cure ulcerative colitis or replace accepted methods of treatment, but it appears to be a useful therapeutic adjunct, more effective than cortisone and less potent than ACTH.

(2) The advantages of hydrocortisone, in comparison with corticotropin, are easy oral administration and fewer, less severe side effects.

(3) The principal indications for hydrocortisone in ulcerative colitis may be as adjunct therapy in the moderately severe disease and as prolonged maintenance treatment following the improvement initiated by corticotropin.

### *References*

1. KIRSNER, J. B. & W. L. PALMER. 1954. Ulcerative colitis consideration of its etiology and treatment. *J. Am. Med. Assoc.* **155**: 341.
2. KIRSNER, J. B. & W. L. PALMER. 1951. Effect of corticotropin (ACTH) in chronic ulcerative colitis observations in forty patients. *J. Am. Med. Assoc.* **147**: 541.
3. KIRSNER, J. B. & W. L. PALMER. 1954. Ulcerative colitis: therapeutic effects of corticotropin (ACTH) and cortisone in 120 patients. *Ann. Internal Med.* **41**: 232.
4. WEST, H. F. 1954. Purified ACTH gel—control of therapy in rheumatoid patients. *Ann. Rheumatic Diseases.* **13**: 56.
5. BOLAND, E. W. & W. D. HEADLEY. 1952. Compound F used orally in patients with rheumatoid arthritis. *J. Am. Med. Assoc.* **148**: 981.
6. BOLAND, E. W. 1952. Clinical use of cortisone, hydrocortisone and corticotropin. *J. Am. Med. Assoc.* **150**: 1281.
7. WARD, L. E., H. F. POLLEY, C. H. SLOCUMB & P. S. HENCH. 1953. Cortisone in treatment of rheumatoid arthritis. *J. Am. Med. Assoc.* **152**: 119.
8. WOLFSON, W. Q. 1953. The three subtypes of pituitary adrenocorticotropin. *Arch. Internal Med.* **92**: 108.

## THE USE OF HYDROCORTISONE IN CANCER\*

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Cortisone and ACTH will induce suppression of growth and regression of several types of human neoplasms.<sup>1, 2</sup> These hormones are useful for the palliative treatment of chronic lymphatic leukemia, acute leukemia, breast carcinoma, lymphosarcoma, multiple myeloma, prostatic carcinoma, and Hodgkin's disease. Hormone-induced remissions are transient, and relapse usually occurs promptly when the hormone is withdrawn. Sustained remissions require continuous hormone treatment although, with most neoplasms, refractoriness to the effects of the hormone develops and treatment is no longer effective. Pharmacological doses of hormone are usually required to induce remissions. Prolonged administration of large doses of cortisone produces Cushing's syndrome, but most patients tolerate this condition well, even when treatment is continued for several years.

*Chronic lymphatic leukemia.* Most patients with chronic lymphatic leukemia respond to cortisone or hydrocortisone as evidenced by marked shrinkage of enlarged lymph nodes, liver, and spleen. Regrowth of lymphoid tumor masses usually occurs within a few weeks if the hormone is stopped. Readministration of the hormone will again produce marked shrinkage of the lymphoid tumors. Refractoriness to the effects of cortisone does not appear to develop, even after periods as long as four years. Continuous administration of cortisone in doses 100 to 300 mg. per day is necessary to sustain suppression of the lymphoid tumor growth.

Most patients will obtain improvement in the hematological picture during hormone treatment. There is a rise in the reticulocyte count, followed by better maintenance of hemoglobin levels and, sometimes, a spontaneous rise in hemoglobin to normal levels. Patients with frank hemolytic anemia respond dramatically to cortisone with a prompt remission of the hemolytic phenomenon.

The total leukocyte count rises initially, reaching a peak after about two weeks of treatment, after which the white cell count recedes gradually to below the initial level. There is no significant change in the differential leukocyte count during the first few weeks of treatment. With prolonged treatment, a few patients have shown a return to normal of the total leukocyte count and of the differential white-cell count and a disappearance of the lymphoid infiltration of the bone marrow. With most patients, however, some lymphoid infiltration remains in the bone marrow and in the peripheral blood.

Hemorrhagic manifestations sometimes subside promptly with hormone administration, even though the platelet count does not rise immediately. A rise in platelet count may occur with prolonged therapy.

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Subjective improvement usually occurs promptly and is sustained in most of the patients. This improvement is characterized by a sense of well-being, increased strength, and improved capacity for mental and physical work.

Prolonged administration of large doses of cortisone produces Cushing's syndrome. Most patients tolerate this condition well, and severe complications of Cushing's syndrome have been very infrequent. The most severe complication has been the development of osteoporosis in one patient. This condition was treated successfully by temporarily lowering the cortisone dosage and adding testosterone propionate. We have previously demonstrated<sup>3</sup> that testosterone and cortisone can be given simultaneously without impairing the suppressive effects of cortisone on the lymphoid tumors. The administration of 100 mg. of testosterone propionate three times a week during cortisone therapy is useful in ameliorating some of the undesired physiological effects of the cortisone.

The most common cause of death in patients with chronic lymphatic leukemia is infection. In our early experience with the use of cortisone in this disease, we made the mistake of lowering the cortisone dosage when infection developed, on the assumption that cortisone impaired the host resistance to infection. Several patients promptly succumbed from acute adrenal insufficiency. When prolonged cortisone therapy is undertaken, it is of the utmost importance to maintain or increase the cortisone dosage when infection develops, and to treat the infection in the customary manner.

It may be concluded that cortisone is a useful agent in the treatment of chronic lymphatic leukemia. X-ray therapy and triethylene melamine are also effective in the treatment of this disease, but great care must be used with these modalities to avoid injury to an already impaired bone marrow. Cortisone is the agent of choice in patients with hemorrhagic manifestations and severe thrombopenia.

*Acute leukemia.* Cortisone or hydrocortisone will induce complete remissions in about 50 per cent of children and in a smaller percentage of adults with acute leukemia. The remissions last only for a few weeks or months, however, and the disease returns despite continued administration of the hormone. Cortisone is only useful for very temporary palliation of these patients.

Somewhat more prolonged remissions can be obtained in some patients with the use of the antimetabolites, A-methopterin and 6-mercaptopurine. With the combined or alternating use of these agents and cortisone, survival may be prolonged for periods as long as a year in some patients.<sup>4</sup>

*Breast cancer.* Cortisone, in doses of 200 to 300 mg. per day, will induce symptomatic and objective remissions in 30 to 50 per cent of women with metastatic breast cancer. Symptomatic improvement, without manifest objective change in the neoplasm, can be obtained in an additional 20 to 30 per cent of the cases. The remissions are of short duration, averaging about three months, and relapse occurs despite continued therapy.

Objective improvement was shown by subsidence of hypercalcemia and hypercalciuria in patients with osteolytic tumors, calcification of osteolytic lesions by radiographs, regression of cutaneous, lymph node, and pulmonary



metastases, and shrinkage of a markedly enlarged liver. Symptomatic improvement consisted of relief of pain, improvement in appetite, strength, and sense of well-being.

Cortisone therapy produces worthwhile palliation in many patients with breast cancer. Cortisone however, is not used as the initial form of therapy, except in some critically ill patients. Other forms of endocrine therapy, such as oophorectomy and adrenalectomy<sup>5, 6</sup> will produce longer and more satisfactory remissions in about 50 per cent of women with breast cancer. These modalities of treatment are used first. When relapse occurs following these other types of endocrine therapy, or if the patient fails to respond satisfactorily, cortisone is useful in producing further palliation in some of these patients.

Since it has been shown that adrenalectomy will induce objective remissions in about 40 to 50 per cent of women with breast cancer, it was thought possible that cortisone in maintenance dosage (50 to 75 mg. per day), which is known to suppress the function of the adrenal cortex, might be capable of inducing remissions comparable to those obtained with surgical removal of the adrenals. Of 14 patients treated with 50 to 75 mg. of cortisone per day, only two showed objective improvement which lasted for a few months. Thus, our present experience indicates that small doses of cortisone will not induce remissions comparable to those obtained with adrenalectomy.

*Lymphosarcoma.* Cortisone will induce shrinkage of lymphoid tumor masses in some patients with either follicular lymphosarcoma or reticulum cell sarcoma. In most of these patients, the remissions last only for a few weeks and regrowth of the tumor occurs despite continued hormone therapy. In a few patients with follicular lymphosarcoma, remissions for as long as one year can be obtained with continuous hormone treatment.

X-ray therapy is usually the treatment of choice in this disease. Triethylene melamine is also useful treatment in some patients with disseminated disease. Cortisone is worthy of trial when X-ray therapy no longer produces a satisfactory response or when rapid shrinkage of tumors is desired.

*Multiple myeloma.* Cortisone will induce symptomatic and objective remissions in more than 50 per cent of patients with plasma cell myeloma. There is usually prompt suppression of pain, improvement in strength, appetite, and sense of well-being. Shrinkage of tumor masses, enlarged liver and spleen, and a decreased infiltration of the bone marrow with myeloma cells may be obtained. Other objective evidences of improvement that may occur are improved hematopoiesis, reduction of serum myeloma protein levels, and increased serum albumin.

Remissions from cortisone have lasted as long as 18 months. Refractoriness develops in some patients and the disease again becomes progressive despite continued administration of the hormone in large dosage.

*Prostatic carcinoma.* Symptomatic improvement can be obtained with the use of cortisone in some patients with advanced prostate cancer whose disease is in relapse following orchiectomy and estrogen therapy. The improvement usually lasts only for a few months.

Adrenalectomy will induce symptomatic improvement in some of these pa-

tients, but the improvement lasts for such a short period that this procedure does not seem to be worth while. Hypophysectomy is being explored as a possible means of inducing more worth while remissions.

*Hodgkin's disease.* Some symptomatic and objective improvement can be obtained with the use of cortisone in patients with Hodgkin's disease. The results are minimal and short lasting. X-ray therapy is the treatment of choice in these patients. Triethylene melamine is often useful when the disease becomes disseminated.

*Comment.* Cortisone has been given to a number of patients with a variety of other types of tumors without obtaining any evidence of objective improvement. Cortisone will induce brief periods of symptomatic improvement in some patients, even though there is no evidence of suppression of tumor growth. It is sometimes worth while to use cortisone for symptomatic relief, even though the effects may last but a few weeks.

The mechanism by which cortisone produces suppression of growth and regression of certain human neoplasms is not known. These hormones were first tried in patients with neoplastic disease<sup>7</sup> because of their known inhibitory effects on the growth of normal tissues. It is thought that, probably, the same mechanisms are operative in the case of neoplasms as with normal tissues.

The striking, though temporary, remissions which are produced in children with acute leukemia have suggested to us that cortisone may mobilize some essential nutrient substance which produces the remission of the leukemia. There is no substantial evidence, however, that acute leukemia is a deficiency disease, although occasionally a blood transfusion will produce a temporary remission.

### References

1. PEARSON, O. H. & L. P. ELIEL. 1950. Use of pituitary adrenocorticotrophic hormone (ACTH) and cortisone in lymphomas and leukemias. *J. Am. Med. Assoc.* **144**: 1349.
2. WEST, C. D., M. C. LI, J. P. MACLEAN, G. C. ESCHER & O. H. PEARSON. 1954. Cortisone-induced remissions in women with metastatic mammary cancer. *Proc. Am. Assoc. Cancer Research.* **1**: 51-52.
3. PEARSON, O. H. & L. P. ELIEL. 1950. Experimental studies with ACTH and cortisone in patients with neoplastic disease. *Rec. Prog. in Hormone Research.* Academic Press. New York, N. Y. **6**: 363-416.
4. BURCHENAL, J. H. & M. L. MURPHY. 1954. The management of acute leukemia in childhood. *N. Y. State J. Med.* **54**: 3362-3365.
5. PEARSON, O. H., C. D. WEST, M. C. LI, J. P. MACLEAN & N. TREVES. 1955. Endocrine therapy of metastatic breast cancer. *Arch. Internal. Med.* In press.
6. PEARSON, O. H., C. D. WEST, V. P. HOLLANDER & N. TREVES. 1954. Evaluation of endocrine therapy for advanced breast cancer. *J. Am. Med. Assoc.* **154**: 234-239.
7. PEARSON, O. H., L. P. ELIEL, R. W. RAWSON, K. DOBRINER & C. P. RHOADS. 1949. ACTH- and cortisone-induced regression of lymphoid tumors in man—a preliminary report. *Cancer.* **2**: 943-945.

## THE USE OF CORTICOIDS IN ASSOCIATION WITH ANTIBIOTICS IN THE MANAGEMENT OF UNUSUALLY SEVERE INFECTIONS\*

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Two sets of observations exist that superficially appear to be in conflict, in regard to the place of adrenocortical steroids of the cortisone type in the total picture of resistance to infection.

It has been known for many years that the patient with Addison's disease, and adrenalectomized animals, are hypersusceptible to infection. In the era prior to cortisone, the average Addisonian patient maintained on desoxycorticosterone was subject to relatively frequent hospitalization, as the result of rapid progression of diseases which frequently began as simple upper respiratory infections. Such individuals usually required large amounts of whole adrenocortical extract to prevent Addisonian crisis, and/or death from infection *per se*. In contrast, the Addisonian patient, maintained on optimal amounts of cortisone or hydrocortisone (with or without desoxycorticosterone, as indicated in the individual case) appears to be no more susceptible to infection than the average non-Addisonian individual. Essentially the same situation exists in adrenalectomized animals. From the preceding set of observations, therefore, it is apparent that cortisone-like steroids play some essential role in the over-all composite of resistance to infection.

Other observations on experimental animals, pretreated with pharmacologic amounts of adrenal steroids, receiving no antibiotics, and then inoculated with pathogenic organisms, indicate beyond question that, under suitable experimental conditions, the corticoid-treated animals have a much higher mortality than do control animals. At first glance, this observation seems to be directly opposed to those noted in the preceding paragraph. If, however, one carefully examines the known physiological and pharmacological effects of the cortisone-like steroids, the picture comes into focus.<sup>1</sup>

It may be well to consider the evolution of our knowledge regarding the place of corticotropin, cortisone, and related substances in clinical medicine. In 1949, Doctor Hench and his associates electrified and confounded us with their observations regarding the effect of cortisone in patients with rheumatoid arthritis. Later that year, at the "first ACTH conference" sponsored by the Armour Laboratories, Doctor Maxwell Finland reported a chance observation which seemed to us equally as electrifying. A patient seriously ill with pneumococcus pneumonia (FIGURE 1) inclusive of a positive blood culture, 24 hours after beginning ACTH administration, was essentially clinically well, despite the fact that the positive blood culture persisted until the fifth day. From this and related observations, it became apparent that corticotropin and cor-

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This work was carried on with the aid of many members of the house and attending staffs of Highland Alameda County Hospital, Samuel Merritt Hospital, and Children's Hospital of the East Bay, Oakland, Calif.

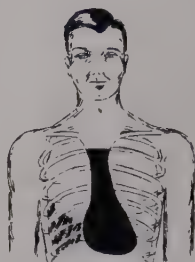
Patients followed have been hospitalized in Highland Alameda County Hospital, Samuel Merritt Hospital, and Children's Hospital of the East Bay, Oakland, Calif.

PATIENT X WITH  
PNEUMOCOCCUS PNEUMONIA  
DAY 1  
NO ACTH



HIGH FEVER  
EXTREME TOXICITY  
POSITIVE BLOOD CULTURE

PATIENT X WITH  
PNEUMOCOCCUS PNEUMONIA  
DAY 2  
PLUS ACTH



NO FEVER  
NO TOXICITY  
POSITIVE BLOOD CULTURE

FIGURE 1. Corticoid-induced eradication of signs and symptoms of pneumonia without inhibition of bacterial growth.

tical steroids when given in sufficient amount would obliterate, largely or completely, the signs and symptoms of severe infectious disease, without in any way inhibiting the growth of organisms. We have called this a "nonspecific antitoxic effect" for lack of a better term. Three possible ways in which such an effect could be produced are shown in FIGURE 2.

One of the most profound and predictable effects of corticotropin and cortisone-like steroids is that of *inhibition of the inflammatory reaction*. It is quite possible that a significant part of the "antitoxic effects" are attributable to this action; *i.e.*, that some of the products of inflammation may be responsible for the systemic toxicity in many infections. Whatever the truth in regard to this may be, there is no question but that the inhibition of the inflammatory reaction, which, under some circumstances can be desirable, can be most dangerous under other conditions. It is this property of corticoids which is largely or wholly responsible for the increased mortality of experimental animals under the conditions noted above; *i.e.*, as the result of inhibition of the inflammatory reaction, an otherwise localized infection becomes disseminated, with resultant death of the animal.

That the inflammatory reaction *can* be a necessary part of the immune machinery is beyond question. That its net results may be most unfortunate is equally true, *e.g.* meningitis and extensive peritonitis.

With the availability of potent antibiotics, all the characteristics of infectious disease, as we knew them a few years ago, have been changed. Lobar pneumonia, a disease which formerly accounted for a large portion of the population of any medical ward, has become almost a rarity. Diseases such as



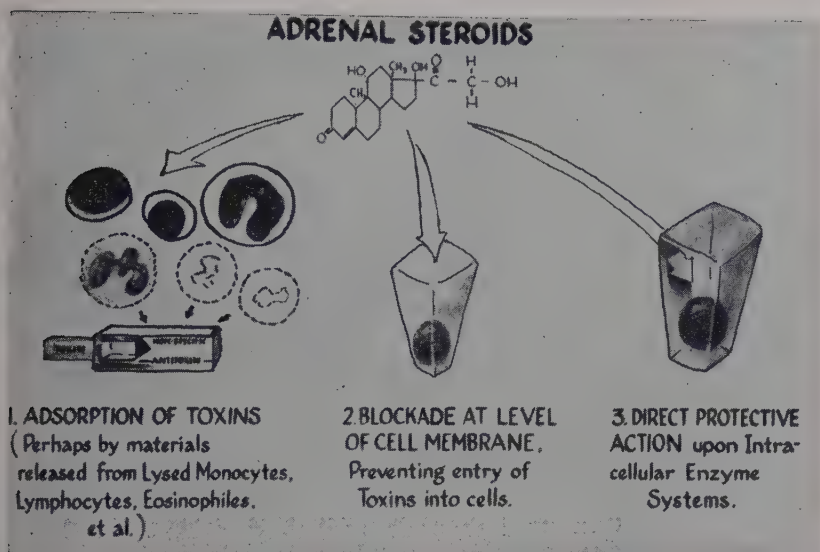


FIGURE 2. Three possible mechanisms of antitoxic effects of adrenal steroids.

pneumococcus meningitis, which formerly resulted in nearly 100 per cent mortality, can now be coped with in a high percentage of cases. Nevertheless, patients still die of infectious diseases *despite* specific antibiotic therapy.

In the Alameda County Hospitals (as in most county hospitals), one sees a rather large number of patients with generalized peritonitis and with other severe infectious conditions, including fulminating meningococcemia, with or without meningitis. Many of these patients enter the hospital in a moribund or near moribund state. Consequently, in 1950, we adopted a working hypothesis to the effect that corticoids, if administered to such patients, would combat shock and toxicity and, consequently, would enable one to keep some of them alive until such time as well indicated antibiotics would have time to take effect. It was further postulated that the proper use of combined hormonal-antibiotic therapy would make possible "early surgery" in peritonitis patients, in whom one would otherwise not dare to undertake such surgery. This hypothesis involved the assumption that corticoids would in no way interfere with the effect of antibiotics, and that, consequently, the antibiotics would more than compensate for any "disseminating effect" of the corticoids. This rationale is shown diagrammatically in FIGURE 3.

The clinical course of one of the very early patients in whom such a technique was used is shown in FIGURE 4. This child entered the hospital more than 48 hours after a probable ruptured appendix. He was critically ill. After two days on combined hormonal-antibiotic therapy, he was essentially asymptomatic. Surgery carried out at this time showed a large amount of thin purulent material, with surprisingly little active inflammatory reaction in either visceral or parietal peritoneum, except at and immediately adjacent to the actual point of rupture of the appendix. After necessary blunt dissection, the

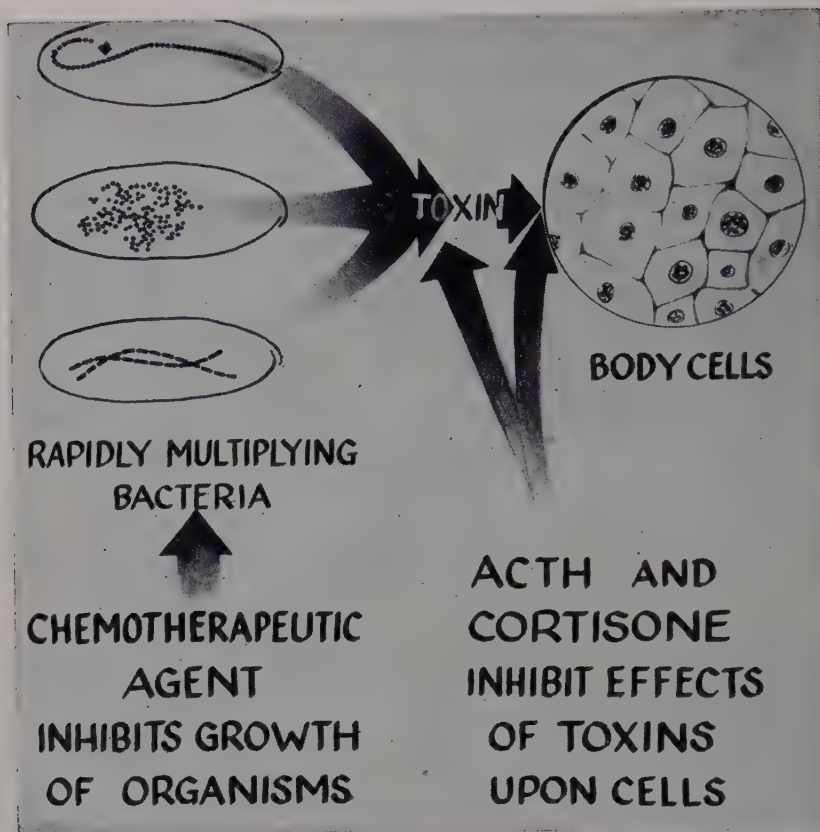


FIGURE 3. Rationale of combined chemotherapy-hormonal therapy in overwhelming states.

appendix was removed, all possible pus removed by suction, and the abdomen closed without drainage. The child made an uneventful convalescence and was discharged in 10 days. Corticoids were administered for a total of seven days. Antibiotics were continued for four days after all corticoids had been discontinued.

In FIGURE 5 is shown the course of a patient who recently received combined therapy. The problem was that of progressive otitis and mastoiditis in conjunction with extreme allergic sensitivity to all indicated antibiotics. Intensive corticoid therapy permitted adequate administration of antibiotics, and was associated with rapid "detoxication" and convalescence.

In FIGURE 6 is shown the course of a child who entered the hospital in a dying condition as the result of combined peritonitis and pneumonitis. Intensive combined hormonal-antibiotic therapy resulted in improvement to a degree which permitted of definitive surgery on the seventh hospital day. Her convalescence over the next nine days was uneventful. As the result of an error, antibiotics were discontinued too soon after discontinuance of corticoids, and

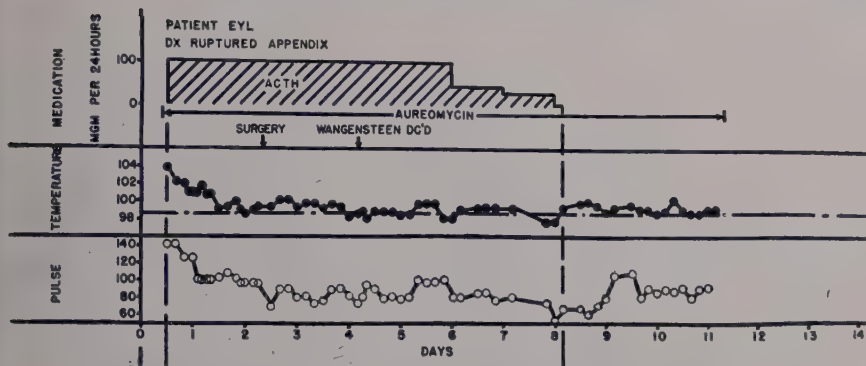


FIGURE 4. Effects of combined hormonal-antibiotic therapy in a child with generalized peritonitis.

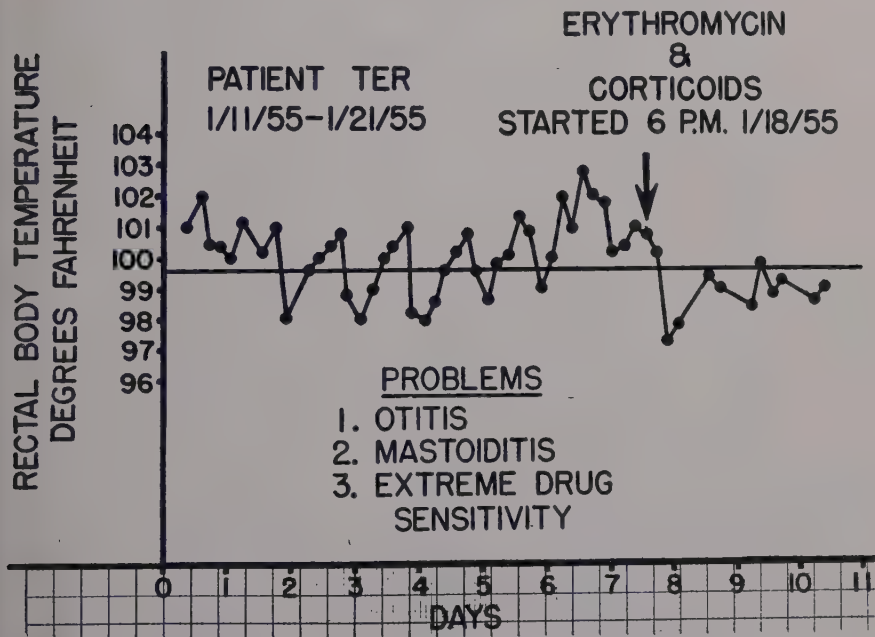


FIGURE 5. Adequate corticoid administration in this patient permitted the use of indicated antibiotics to which the patient had major allergic sensitivity. Full recovery ensued.

evidence of a pelvic abscess and general clinical deterioration ensued. Resumption of combined therapy resulted in disappearance of all signs and symptoms of the disease, and eventual full recovery. The convalescence, however, was markedly prolonged because of the premature discontinuance of specific therapy.

More than 300 seriously or critically ill patients have received combined corticoid-antibiotic therapy in this and affiliated institutions during the past

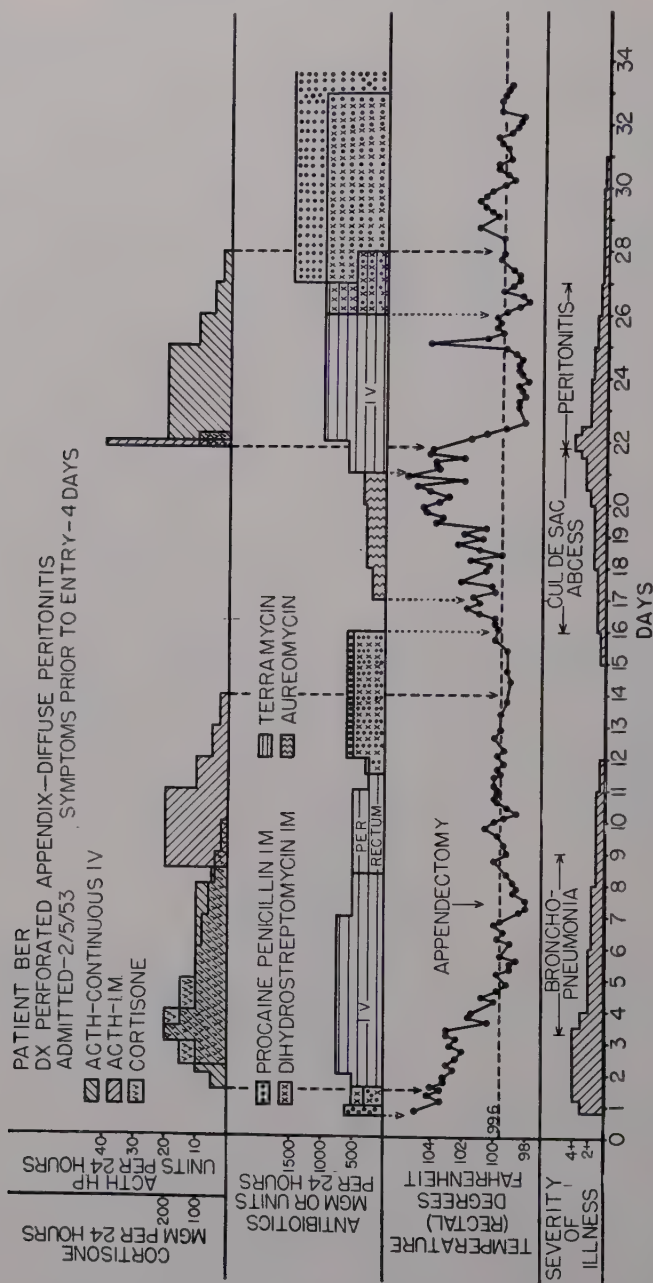


FIGURE 6. Premature discontinuance of antibiotics, particularly in patients with generalized peritonitis, may result in unnecessary and dangerous complications.



five years. On the basis of this experience, we feel that the following statements are in accord with the facts:

(1) Hormonal therapy, in conjunction with properly indicated antibiotic therapy, can reduce mortality and morbidity.

(2) Hormonal therapy injudiciously applied in patients with infectious (or other) conditions can result in fatality.

If both these statements are true, it is obvious that, as is so frequently the case with potent pharmaceutical agents, one has available to him a "two-edged sword." It may, therefore, be well to consider those factors which are essential in: (1) the decision to use or not to use corticoids in infection; (2) the method of application, if the decision is in the affirmative.

#### *Indications for Corticoid Therapy in Patients with Infectious and Related Acute Diseases*

(1) All patients with meningococcus infection. We realize this is a sweeping statement, but believe it is justified for the following reasons:

(a) If time is of the essence in any disease, it is in meningococcemia. The toxin can produce a fatal termination in a surprisingly short period of time.

(b) In no instance in which corticoids have been used properly in patients with meningococcic infections, have we had occasion to feel that any untoward effect has resulted.

(2) All diseases which are characterized by the sum of the following:

(a) Infections for which potent antibiotics are available;

(b) Infections characterized by overwhelming toxicity, *i.e.*, infections in which, either as the result of pre-existing debility, neglect, delay in recognition of the nature of the disease, or unusually virulent organisms, there is major doubt in the mind of the clinician as to whether the patient will survive sufficiently long to permit of the action of indicated antibiotics;

(3) Mumps orchitis;

(4) Unusually severe viral hepatitis;

(5) Shock from any cause, with the sole exception of that resulting from simple blood loss, in individuals for whom blood replacement is immediately available, may also be considered an indication for corticoid therapy.

#### *Relative Contraindications*

Peptic ulcer, active tuberculosis, history of extreme emotional instability, widespread severe chronic eczematoid dermatitis, are all relative contraindications. If the urgency is sufficiently great, corticoids should nevertheless be used. All appropriate measures should be employed; *e.g.*, intensive therapy to maintain constant neutralization of gastric acidity in the ulcer patient, *etc.*

#### *Method of Application of Corticoids in Patients with Infectious Disease*

(1) Carry out all necessary procedures for establishment of a bacteriologic diagnosis at the earliest possible moment.

(2) Immediately after blood and other specimens have been obtained, begin intensive antibiotic therapy on the basis of proven or probable etiology. Such

antibiotic therapy must be continued throughout the period of hormonal therapy, and at least for three days thereafter. In the case of severe peritonitis, it is probably well to administer antibiotics at least for one week after the last dose of corticoids.

(3) Coincident with the administration of antibiotics, administer intravenous ACTH, intramuscular cortisone acetate, intravenous free hydrocortisone, the latter by infusion; and also by "stat" intravenous injection of free hydrocortisone in propylene glycol, or an aqueous solution of hydrocortisone hemisuccinate.\* Blood levels obtained with this material are shown in FIGURE 7.

(4) Over a period of four to seven days, give gradually diminishing amounts of corticoids as shown in FIGURE 8. ACTH is always administered one day longer than cortisone to eliminate any concern about adrenal atrophy.

One may summarize the principles involved as follows:

- (1) Very intensive initial corticoid therapy to combat shock and/or toxemia.
- (2) Thoroughly adequate antibiotics to eliminate the infection, and to counteract adequately the tendency to dissemination of infection, associated with corticoid therapy.

(3) Discontinuance in a step-wise fashion of the corticoids, *as rapidly as is compatible with the patient's clinical status*. Probably more than 90 per cent of our patients receive the four-day, rather than the seven-day program. An occasional patient will require corticoid support for a period longer than seven days.

In the case of mumps orchitis and viral hepatitis, specific antibiotics are still unknown. There is a fairly abundant literature attesting to the value of corticoids in severe hepatitis. In these patients, particularly those who are comatose at the time therapy is begun, very prolonged corticoid therapy may be required. In mumps orchitis, prompt corticoid therapy can result in prevention of all permanent damage to the testicle. In our small experience with this condition, we have seen nothing suggestive of untoward effects. The four-day program has been used.

### Nutrition

The same general principles apply to the emergency application of corticoids as apply to the nutrition of patients receiving chronic corticoid therapy; namely, high protein, adequate calories, low sodium, and high potassium. In many of the patients with severe infections, adequate nutrition can not be obtained. In a patient whose nutrition has been good prior to the onset of infection, and in whom the four-day program is sufficient, this does not represent a major problem; *i.e.*, the administration of glucose plus essential vitamins and mineral supplements will be adequate. Our standard initial infusion consists of 5 per cent glucose in distilled water, 2 gm. of potassium chloride, the indicated amount of antibiotics, 20 units of ACTH, and 100 mg. of hydrocortisone. This is administered at the rate of 100 cc. per hour.

\* The free hydrocortisone in propylene glycol was supplied through the kindness of Doctor Edward Henderson of the Schering Corporation; the hydrocortisone hemisuccinate was supplied by Doctor C. J. O'Donovan, The Upjohn Company, Kalamazoo, Mich. 100 mg. of the former is contained in a volume of 4 cc.; 133.7 mg. of the latter is contained in one ampule vial, (equivalent to 100 mg. of hydrocortisone.) The addition of 2 cc. of sterile distilled water results in a solution which is chemically stable for several hours.

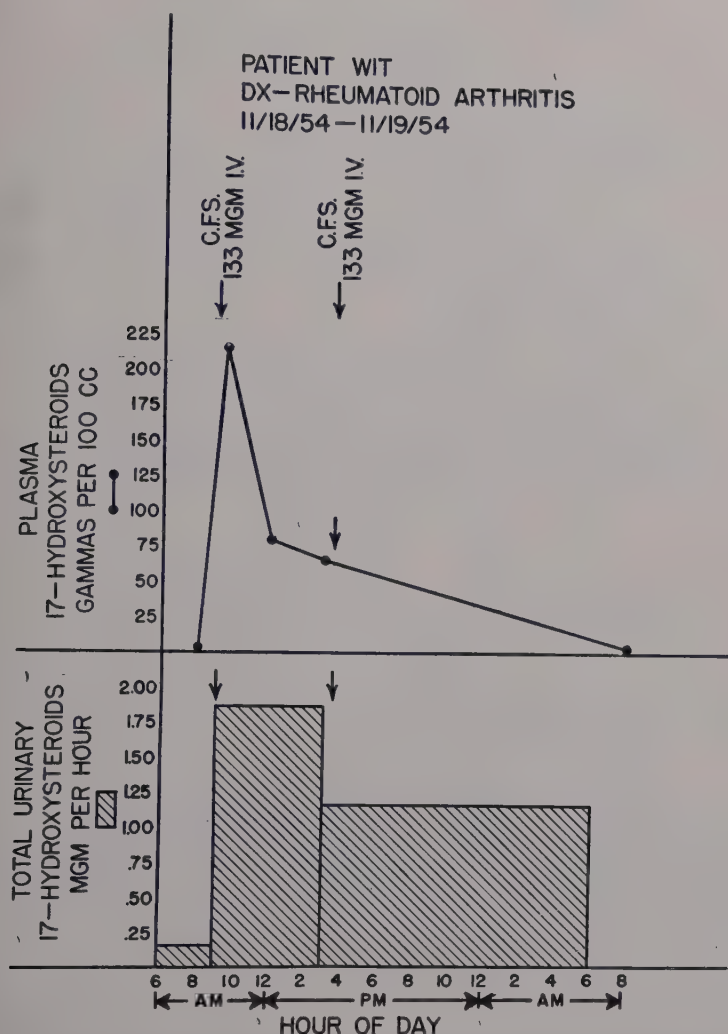


FIGURE 7. High blood levels of 17-hydroxycorticoids are obtained following the administration of hydrocortisone hemisuccinate or hydrocortisone in propylene glycol. Therapeutic effects can be demonstrated within one hour. This amount of the former material may be administered in a two cc. volume of aqueous solution; the latter in four cc. of propylene glycol.

When the patient is unable to take food by mouth, but where tube-feeding is permissible, a small plastic tube is inserted into the stomach or upper small intestine, and a high protein, high potassium, low sodium, adequate calory formula is administered hourly throughout the 24 hours. The desalted milk preparation, Lonalac\*, supplemented with essential vitamins and with potassium has proved to be most useful in this regard.

\* Grateful acknowledgment is made to Mead Johnson and Company, Evansville, Ind., for adequate supplies of this material for clinical evaluation.

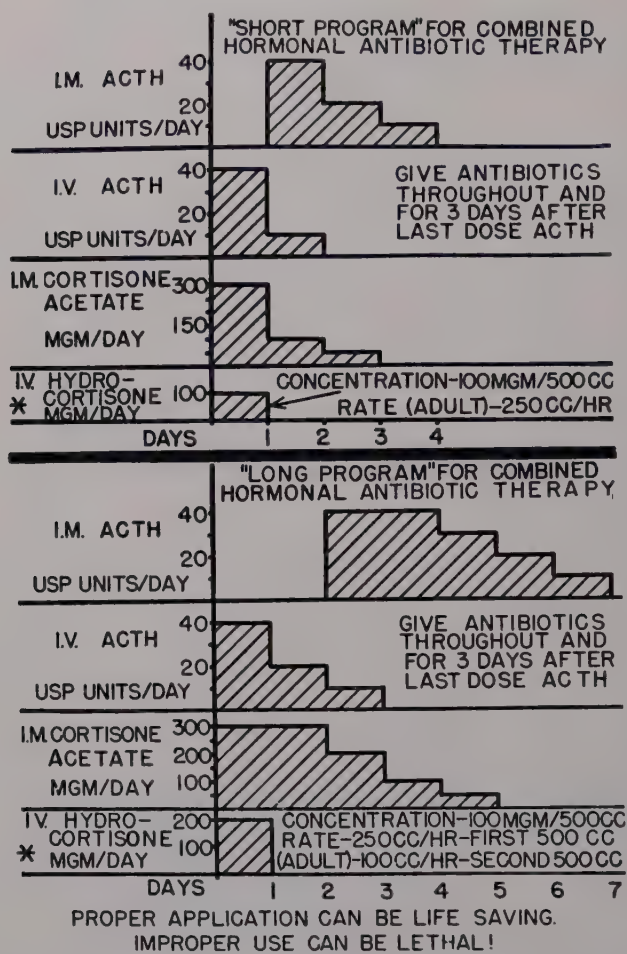


FIGURE 8. "Standard" short-term and long-term program for combined hormonal-antibiotic therapy.

\* In addition to free hydrocortisone by infusion, free hydrocortisone in propylene glycol, or hydrocortisone (as the hemisuccinate) may be given "stat"; 133.7 mg. of the latter material is dissolved in 2 cc. of water immediately before administration.

### Discussion

As indicated, over a period of approximately five years, in the institutions in this city in which this work has been carried out, combined hormonal-antibiotic therapy in patients with severe infections has evolved from the investigative phase to a routine clinical phase. That such therapy can be of very great value, we believe is beyond any question. That it should not be used lightly or indiscriminately is equally true. Particularly to be borne in mind is one fact—that *corticoids can cause one's clinical judgment to become worthless.*



The first patient treated in this hospital is as striking an illustration of this statement as any whom we have seen. He was admitted four to five days after a ruptured appendix, in critical condition, with a rigid, completely silent abdomen. Twenty-four hours after the institution of hormonal therapy, he was sitting up in bed, playing. Not only did he have active peristalsis, but he had had a spontaneous stool. Surgery confirmed the diagnosis. Lack of recognition of the characteristic effects of the corticoids could have resulted in delay of surgery, in association with continued corticoid administration, and almost certain fatality. At postmortem, one would have found miliary abscesses in most of the organs and tissues of the body. We have, in a few tragic instances, seen this picture in patients in other institutions, where such misapplication of these potent therapeutic agents has been involved.

At the risk of being tedious, we must repeat, therefore, that *the decision to use corticoids in a patient with a surgical condition, at the same time involves the decision to carry out definitive surgery at the earliest moment the patient becomes a good operative risk.* Corticoid therapy should be discontinued as soon after surgery as the patient's condition will permit (see above).

### Summary

On the basis of five years' experience with more than 300 patients, it has become apparent that the judicious use of corticoids in conjunction with antibiotic therapy, in patients with severe medical and surgical infections, can result in diminution in morbidity and mortality.

Improper administration can and will result in increased and unnecessary mortality.

### Reference

1. JAHN, J. P., L. BOLING, T. R. MEAGHER, H. H. PETERSON, G. THOMAS, B. M. FISHER, A. E. THILL, W. A. LEOVY, H. E. BALCH & L. W. KINSELL. 1954. The combination of ACTH-cortisone-hydrocortisone with antibiotics in the management of overwhelmingly severe infections. Theory and practice based on three years' experience. *J. Pediat.* **44**: 640-657. (This source lists the critical literature on which these and other statements in this paper are based.)

# ALLERGIC REACTIONS TO THERAPEUTIC AGENTS: TREATMENT WITH HYDROCORTISONE

By Lawrence E. Shulman

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## *Introduction*

The beneficial influence of cortisone and corticotropin on allergic drug reactions of the serum-sickness type has been repeatedly documented<sup>1-6</sup> and, in our opinion, represents one of the most useful clinical applications of adrenocortical steroid therapy. The inciting agent can often be recognized and withdrawn. In most cases the response to treatment is prompt and complete. Usually only a short course of treatment is necessary and, consequently, the hazards are few and the treatment inexpensive. These hormones are, of course, only recommended for patients who have not responded to less hazardous measures such as antihistaminics or epinephrine.

Both corticotropin and cortisone have been found effective in suppressing these reactions, but the most impressive results have occurred with oral cortisone.<sup>5</sup> The individual dose and the frequency of its administration both depend on the severity of the reaction. In the average case 100 mg. of oral cortisone is given initially, followed by 50 mg. every two to six hours until the reaction has largely subsided, at which point a rather rapid dose reduction may be instituted. Relapses, which occur in some cases, are usually mild, short-lasting and, in some instances, they respond to antihistaminics.

It has been further shown that, if sufficiently high doses are given, corticotropin or cortisone can block an allergic drug reaction while continuing the administration of the offending allergen.<sup>5, 7</sup> This procedure is risky and should be done only in patients who are critically ill with a serious disease such as subacute bacterial endocarditis or pneumococcal meningitis, where penicillin sensitivity may appear and no other satisfactory antibiotic therapy is available.

## *Results*

Hydrocortisone, or compound F, has been given to a small number of patients with severe drug allergies. Our study of the effectiveness of this hormone in drug reactions is still in progress. This is a preliminary report. The results thus far, however, have been instructive. The offending agents have included penicillin, tetanus antitoxin, thiamin, pilocarpine, and ACTH. Both the free alcohol and the acetate have been administered by various routes.

The response to the intramuscular administration of hydrocortisone acetate was slow in the one patient in whom it was tested. This experience, plus the generally-reported inefficacy of this substance when injected into muscle, has discouraged further trial. We have not tested the free alcohol of hydrocortisone intramuscularly.

When the free alcohol of hydrocortisone was given by mouth, however, the responses were as prompt and as complete as those obtained with oral cortisone acetate. One patient so treated (FIGURE 1) was:

R. W. (J.H.H. No. 269042), a 40-year old man with a moderately severe reaction to penicillin, manifested by fever ( $101^{\circ}$  F.), generalized urticaria, and severe acute immobilizing arthritis, particularly of the wrists and metacarpo-phalangeal joints, who was given hydrocortisone according to the regimen outlined above for cortisone, 100 mg. initially, followed by 50 mg. every four hours. The response was dramatic. Three hours after the first dose, the urticaria had disappeared. One hour later, the joints were no longer swollen or painful, and the temperature had fallen to within normal limits. In all,

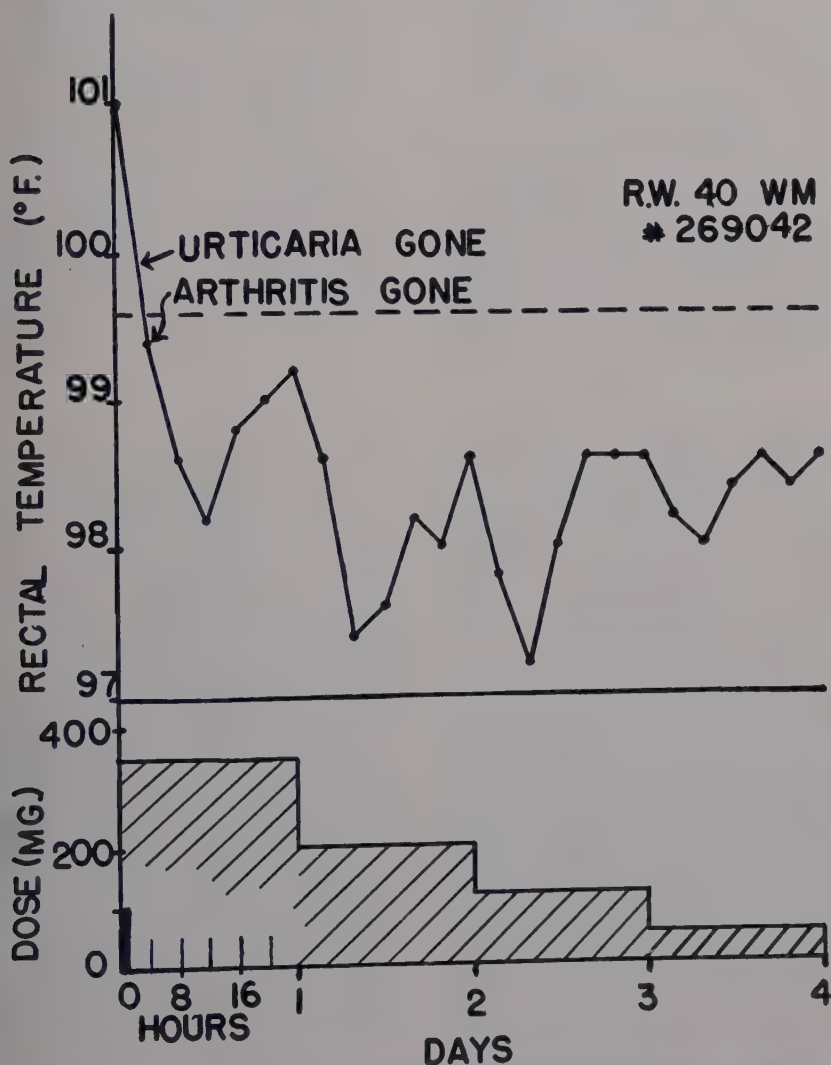


FIGURE 1. R. W. (J. H. H. No. 269042). Hypersensitivity to penicillin; treatment with oral hydrocortisone (free alcohol).

he received 730 mg. over four days and there was no recurrence. This response to hydrocortisone was as striking as those seen with oral cortisone.

Data which will provide an accurate comparison between the clinical potency of two or more steroids are often difficult to obtain. To procure them one needs to compare the results in large groups of cases, or to be able to carry out paired, or matched, clinical trials in the same patient at similar stages of disease activity. Moreover, such a comparison is more readily carried out when the manifestations of the reaction are easily observed, as in the urticaria and angioneurotic edema of drug allergies, than in diseases with more occult manifestations. To test the efficacy of a therapeutic agent, however, it is also preferable to have an illness that is expected to continue if untreated. In this sense, many allergic reactions to drugs are far from ideal. Some drug reactions, however, appear to run a protracted, unremitting, progressive course and, hence, may be very suitable for such a comparative testing. Such a patient was:

A. M. (J.H.H. No. 425518), a 47-year-old woman who, 10 days after receiving oral penicillin for a poorly defined variety of arthritis, developed burning and itching about the mouth, and then a severe maculopapular eruption which spread over her entire body during the six weeks before admission to the hospital. On admission her skin appeared as a thin, dry, scaling, leathery sheet floating on top of a large collection of subcutaneous fluid (FIGURE 2). Clear serum exuded from the cracks in the intertriginous areas. She also had fever, generalized lymph node enlargement, and three-plus pitting edema to the mid-tibiae. Laboratory abnormalities included leukocytosis (25,400 per mm.<sup>3</sup>), striking eosinophilia (2,075 per mm.<sup>3</sup>), thrombocytopenia (74,000 per mm.<sup>3</sup>), and hypoalbuminemia (2.8 gm. per 100 ml.). She was given oral hydrocortisone (free alcohol) starting at 100 mg. and then 50 mg. every four hours for two days with stepwise dose reduction thereafter. In all, she received 2,190 mg. over 17 days. Within 24 hours, she was afebrile, the skin was less red, and the oozing had almost ceased. By the fourth treatment day she had shed all of her parchment-like shell, except over the hands and feet, unveiling a smooth flexible re-epithelialized skin surface (FIGURE 3). The hydrocortisone, however, did not prevent the development of dry gangrene on the tenth treatment day on the second and fifth digits of the right hand, which later dropped off (FIGURE 4).

One week after stopping the hydrocortisone, fever, dermatitis, and eosinophilia reappeared and, over the next five weeks, the skin became progressively more exfoliative until it appeared much like it did on admission before hydrocortisone therapy (FIGURE 4). As may be seen, the patient had lost virtually all scalp hair.

She was then given a course of oral cortisone identical to the previous course of oral hydrocortisone. The clinical response to cortisone was equally as dramatic as that to hydrocortisone. Within 24 hours she was afebrile and less distressed by the itching and, on the third treatment day, the skin was again smooth and flexible (FIGURE 5).

Again during the first week after discontinuing cortisone, there was a prompt relapse with a red, scaling dermatitis and eosinophilia. She was then





FIGURE 2. A. M. (J. H. H. No. 425518). Hypersensitivity to penicillin; before treatment with oral hydrocortisone.



FIGURE 3. A. M. (J. H. H. No. 425518). Hypersensitivity to penicillin; ninth day of treatment with oral hydrocortisone.

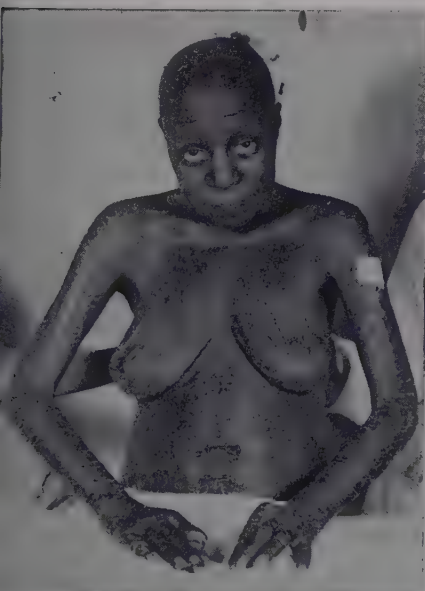


FIGURE 4. A. M. (J. J. H. No. 425518). Hypersensitivity to penicillin; before treatment with oral cortisone.

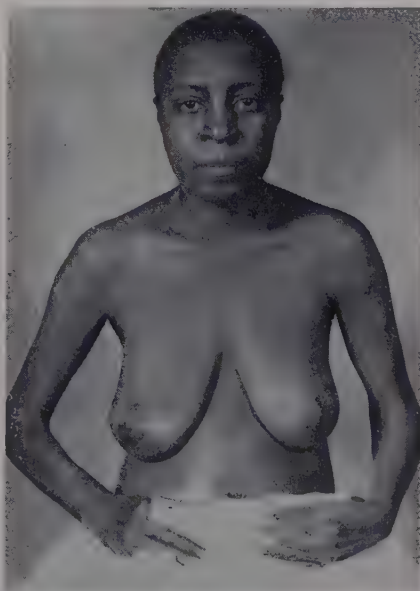


FIGURE 5. A. M. (J. J. H. No. 425518). Hypersensitivity to penicillin; eighth day of treatment with oral cortisone.

re-treated with an eight-week course of oral cortisone, with similarly favorable results. The scalp hair grew back over the next five months, and the allergic reaction did not recur over the next three years.

The eosinophil responses to hydrocortisone and cortisone were both very sharp, being slightly more rapid to hydrocortisone (FIGURE 6). With hydrocortisone, the eosinophil count rose at 2 hours to 2,431 per mm.<sup>3</sup>, dropped to 253 at 4 hours after the initial dose of 100 mg., to 99 at 6 hours, and to 33 at 8 hours. With cortisone, the eosinophils decreased from 1200 to 759 at 4 hours and, as with hydrocortisone, to 33 at 8 hours. With both hormones, eosinopenia persisted throughout treatment, and eosinophilia returned one week after treatment.

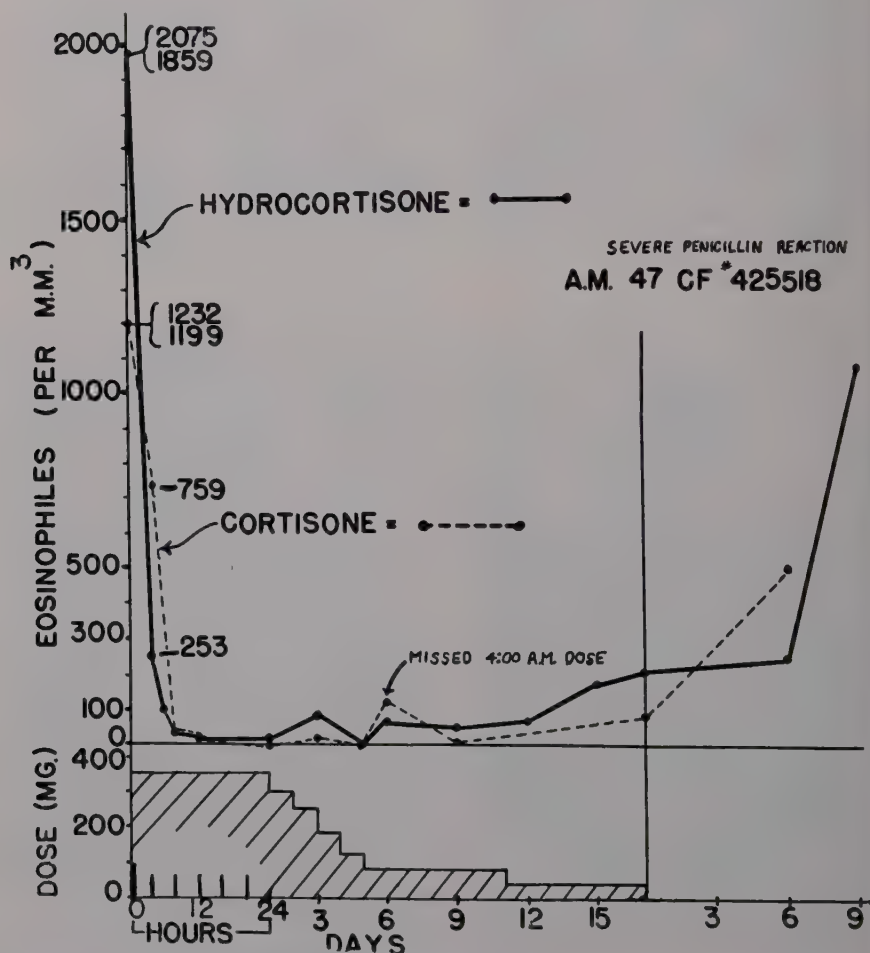


FIGURE 6. A. M. (J. H. H. No. 425518). Hypersensitivity to penicillin; eosinopenic responses to oral hydrocortisone or oral cortisone.

In some cases, an initial dose of hydrocortisone much below 100 mg. may not be optimally effective. This was suggested in:

B. C. (J.H.H. No. 597638), a 43-year-old white woman who, after repeated intramuscular injections of thiamin, developed severe urticaria and angio-neurotic edema. On 50 mg. hydrocortisone orally at two-hour intervals, there was less pruritus, but new urticarial lesions continued to appear. When the dose of hydrocortisone was raised to 100 mg., there was greater relief from the pruritus, and the urticaria disappeared promptly.

It would appear also that, in some instances, the doses of hydrocortisone need to be administered as frequently as doses of cortisone. This was shown in the case of:

R. S. (J.H.H. No. 650936), a 23-year-old laboratory technician, who developed generalized urticaria and polyarthrititis 10 days after receiving tetanus antitoxin and penicillin for lacerations incurred in an automobile accident. On hydrocortisone acetate orally, 80 mg. initially and 40 mg. every four hours thereafter, there was prompt and maintained clearing of the entire allergic reaction. When the interval between the 40 mg. doses was lengthened from four to six hours, however, the urticaria and joint pains reappeared. On resuming therapy at four-hour intervals, the reaction again cleared promptly and completely, and did not recur when the dose was reduced to 30 mg. every four hours.

Satisfactory responses may occur with low doses of oral hydrocortisone, as in this patient (FIGURE 7):

E. C. (J.H.H. No. 259036), a 27-year-old Negro male with acute rheumatic fever, was given a three-week course of both pork and beef ACTH intrave-

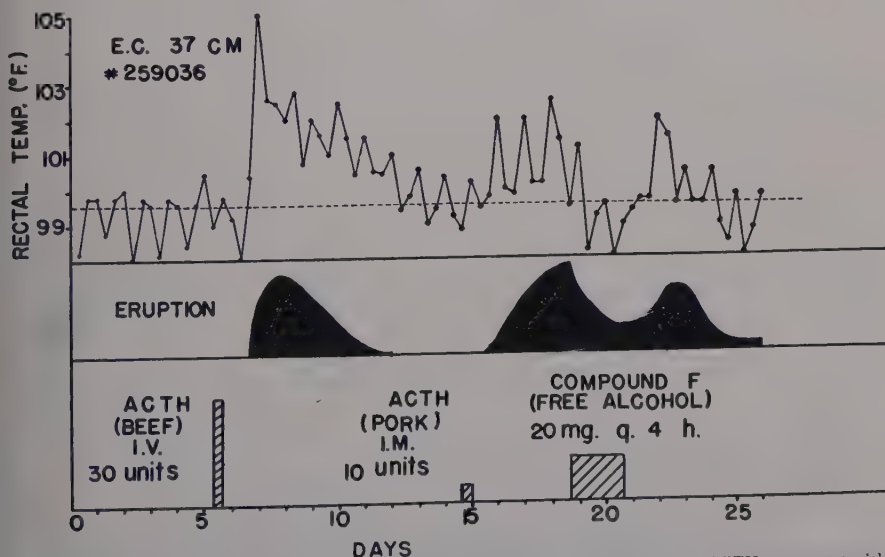


FIGURE 7. E. C. (J. H. H. No. 259036). Hypersensitivity to beef ACTH and pork ACTH; treatment with oral hydrocortisone (free alcohol).

nously. Eighteen days later, when his rheumatic fever recurred, he was again given beef ACTH, 30 units intravenously. Sixteen hours after the end of the infusion he had a shaking chill, the temperature rose sharply to 105° F. (R), and an erythematous maculopapular eruption appeared. The temperature returned to normal and the eruption faded over the next week. The patient was then given a test dose of 10 units pork ACTH intramuscularly and, again, had a delayed reaction with fever (102° F.) and a more violent eruption. He was given hydrocortisone free alcohol 20 mg. orally every four hours for two days. Forty minutes after the first dose, there was less itching. The temperature was normal in eight hours. After 24 hours the eruption had faded and the eosinophil count had dropped from 352 to 44 per mm.<sup>3</sup> Two days after the last dose of hydrocortisone, fever, pruritus and erythema reappeared. There was gradual improvement over the next three days.

On the other hand, in other cases, doses of this magnitude may be unable to prevent the emergence of an allergic drug reaction. This was the situation in the following case:

W. B. (J.H.H. No. 669231), a 62-year-old sales manager, with a severe atopic dermatitis involving chiefly the face, neck, and flexor surfaces of elbows and knees, had been well-controlled for several months on maintenance therapy with oral hydrocortisone-acetate, 100 mg. daily. In December 1954, he began to see "halos" around the headlights of oncoming automobiles. He consulted his ophthalmologist, who found the ocular tension to be elevated and prescribed pilocarpine eye drops. Two days later, the "halos" had gone but the eyelids began to itch. Thinking that his ocular difficulty was becoming worse, he continued to instill the pilocarpine drops. When seen by us 10 days later, the atopic dermatitis was, as previously, quiescent, but over the eyelids and the surrounding skin there was an extremely pruritic scaling erythema. The patient looked as if he were wearing a red mask at a masquerade ball. After discontinuing the pilocarpine and raising the hydrocortisone acetate to 160 mg. daily for three days, this local reaction disappeared and did not recur when the dose was again reduced to 100 mg. daily.

Theoretically, the most rapid and reliable means of administering adrenal corticoids is by the intravenous route. Consequently, the recently available preparations of hydrocortisone for intravenous administration may have special application in severe fulminating drug reactions and in those patients unable to take oral medication, one reason for which may be the nausea and vomiting of serum sickness. The results in the first patient treated with intravenous hydrocortisone were very disappointing:

A. G. (J.H.H. No. 672349), a 16-year-old white boy, had a severe reaction to either tetanus antitoxin or penicillin with fever (102° F.), urticaria and angioneurotic edema, and obtained no response to 100 mg. of hydrocortisone acetate given intravenously over 75 minutes. A dose of 100 mg. of cortisone acetate, given orally one hour later, was similarly ineffective. The patient was then placed on 50 mg. cortisone every four hours, and had such a slow response, fever and urticaria persisting until the fourth day of therapy, that it was hard to ascribe to the cortisone any therapeutic effect.



The second patient, however, had a very dramatic response to intravenous hydrocortisone (FIGURE 8).

J. S. (J.H.H. No. 157288), a 26-year-old white male, was admitted with a severe reaction to either tetanus antitoxin or penicillin and consisting of fever ( $102^{\circ}$  F.), severely pruritic, widespread urticaria and lymphadenopathy. One half hour after the start of the one-hour infusion of 100 mg. hydrocortisone acetate, the pruritus stopped. At the end of the infusion some of the urticarial lesions had disappeared and others were smaller. Three hours later, they had all disappeared. The temperature dropped  $1.0^{\circ}$  F. (to  $101.0^{\circ}$  F.) by the end of the infusion, and to within normal limits ( $99.4^{\circ}$  F., rectal) three hours later. Without further therapy the reaction did not recur.

Intravenous hydrocortisone acetate has been given concomitantly with whole blood to three patients who had previously had repeated highly febrile trans-

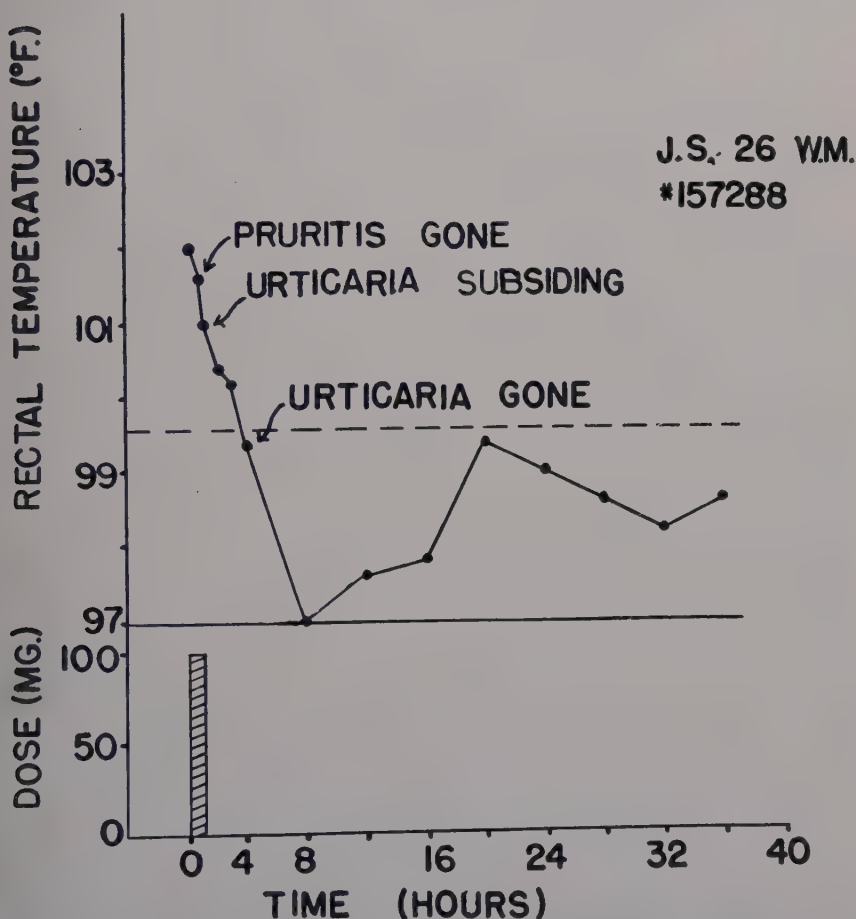


FIGURE 8. J. S. (J.H.H. No. 157288). Hypersensitivity to either tetanus antitoxin or penicillin; treatment with a single one-hour intravenous infusion of 100 mg. hydrocortisone acetate.

fusion reactions in an attempt to block them. The technique used was to time the intravenous therapy so that the hydrocortisone infusion, 100 mg. in each instance, started one hour before and ended one hour after the blood transfusion. During the simultaneous administration of blood and hydrocortisone, these patients remained afebrile or had only a slight fever. We hesitate, however, to assign a therapeutic effect without further confirmation of these findings because these patients were severely ill with serious hematological disorders and had periods of moderately high fever at times other than when blood was given. Moreover, at the time of chills and fever during the transfusions, no other manifestations, such as urticaria, appeared to support the concept that the fever was on an allergic basis.

### *Discussion and Summary*

The responses to oral cortisone in some patients with allergic reactions to drugs have been so impressive that it is difficult to see in what way they could be improved. From the preliminary experience reported here, oral hydrocortisone seems capable of achieving equally impressive responses. As one might expect, no qualitative differences were observed between the effects of cortisone and those of hydrocortisone.

These cases also serve to emphasize once again the quantitative nature of this inhibition. Some patients seem to require moderately high doses before obtaining an adequate response. Others may obtain similar responses with smaller doses. In still others, the frequency of administration appears to be important. Finally, patients on hormone doses sufficient to suppress the manifestations of their underlying disease, which may be allergic, and also sufficient to inhibit drug reactions in other patients, may acquire a superimposed drug hypersensitivity.

From this limited experience, a quantitative comparison between the amounts of oral hydrocortisone and cortisone required to elicit equivalent responses can not be drawn accurately. Our impression is that the dose, frequency of administration, and over-all duration of treatment are approximately the same for the two compounds. In other allergic diseases<sup>8-11</sup> and in the experimental induction of hypersensitivity in laboratory animals,<sup>12, 13</sup> the relative hydrocortisone potency has been variously reported, ranging from twice the strength of cortisone to being no more potent.

It is frequently stated, in general terms, that the dose requirements of hydrocortisone are two thirds that of cortisone. Perhaps no fixed ratio of hydrocortisone to cortisone dose requirements can be prescribed that will apply to all diseases with which these steroids are concerned. The ratio of relative clinical potency, when tested under the conditions of replacement therapy, as in Addison's disease, may differ from the relative potency obtained in patients with a chronic inflammatory disease, such as rheumatoid arthritis and, in turn, neither ratio may be the same as that observed in patients with an acute inflammatory process, such as serum sickness. There is a far wider divergence between the various ratios of clinical potencies, hydrocortisone to cortisone, and the relative antiphlogistic potencies of hydrocortisone to cortisone, 78.4 to 1, as determined in mice by Dougherty.<sup>14</sup>

The findings in one patient with pronounced eosinophilia accompanying a severe drug reaction that the eosinopenic response to hydrocortisone was slightly faster than that to cortisone are in substantial agreement with those of Cochrane, Jahn, Foreman, and Kinsell<sup>15</sup> for their two patients, one with atopic dermatitis and the other with asthma. The slightly delayed eosinopenic response to cortisone may represent the time needed for the conversion of cortisone to hydrocortisone or some other differential metabolic feature.

Nor can conclusions now be drawn concerning the efficacy of intravenous hydrocortisone. The patient not responding to this therapy also had one of the poorest responses to oral cortisone that we have seen. The reason for this is not clear. The patient did not appear to be any more severely ill than many of the patients who had responded promptly to a similar course of oral cortisone, nor was he sicker than the patient who had a dramatic response to intravenous hydrocortisone, given in the same manner.

### References

- CAREY, R. A., A. M. HARVEY, J. E. HOWARD & P. F. WAGLEY. 1950. The effect of adrenocorticotrophic hormone (ACTH) and cortisone on drug sensitivity reactions. *Bull. Johns Hopkins Hosp.* **87**: 354.
- SCHWARTZ, E. 1951. Effect of cortisone on severe reactions due to penicillin hypersensitivity. *Am. Acad. Allergy, Proc. Meet. Feb. 6th.* *J. Allergy.* **22**: 94.
- FEINBERG, S. M., T. B. DANNENBERG & S. MALKIEL. 1951. ACTH and cortisone in allergic manifestations: therapeutic results and studies on immunological and tissue reactivity. *J. Allergy.* **22**: 195.
- FRIEDLÄNDER, S. & A. S. FRIEDLÄNDER. 1951. Oral cortisone therapy in allergic disease. *J. Allergy.* **22**: 291.
- SHULMAN, L. E., E. H. SCHOENRICH & A. M. HARVEY. 1953. Allergic reactions to therapeutic agents: treatment with adrenocorticotrophic hormone (ACTH) or cortisone. *Bull. Johns Hopkins Hosp.* **92**: 196.
- HARVEY, A. M., L. E. SHULMAN & E. H. SCHOENRICH. 1953. The effect of ACTH and cortisone upon allergic diseases. *In The Effect of ACTH and Cortisone upon Infection and Resistance.* :140. *N. Y. Acad. Med. Symp. No. 6.* G. Schwartzman, Ed. Columbia Univ. Press. New York, N. Y.
- BRODEY, M. & C. T. NELSON. 1954. Use of cortisone during penicillin treatment of secondary mucocutaneous syphilis in a hypersensitive patient. *New Engl. J. Med.* **250**: 1069.
- BICKERMAN, H. A., A. L. BARACH & S. ITKIN. 1954. Comparative results of the use of ACTH, cortisone and hydrocortisone in the treatment of intractable bronchial asthma and pulmonary emphysema. *J. Allergy.* **25**: 312.
- ARBESMAN, C. E. & N. B. RICHARD. 1954. Prolonged cortisone and hydrocortisone therapy. *J. Allergy.* **25**: 306.
- SCHWARTZ, E. 1954. Oral hydrocortisone therapy in bronchial asthma and hay fever. *J. Allergy.* **25**: 112.
- FYLES, T. W. & B. ROSE. 1954. The use of oral compound F (17-hydroxycorticosterone) in asthma. *Can. Med. Assoc. J.* **70**: 642.
- SPOERLEIN, M. T. & S. MARGOLIN. 1954. Thymus involution and protection against lethal anaphylactic shock in mice treated with cortisone acetate or hydrocortisone acetate. *Federation Proc.* **13**: 407.
- LONG, D. A. & P. C. SPENSLEY. 1954. Specificity of cortisone and hydrocortisone in depressing sensitivity to tuberculin in guinea pigs. *Lancet.* **1**: 645.
- DOUGHERTY, T. F. & G. L. SCHNEEBELI. 1955. The use of steroids as anti-inflammatory agents. *Ann. N. Y. Acad. Sci.* **61**(2) : 328-348.
- COCHRANE, G. C., J. P. JAHN, N. FOREMAN & L. W. KINSELL. 1953. Evaluation of adrenal steroids administered intravenously, intramuscularly and orally. *J. Clin. Endocrinol.* **13**: 993.

# THE EFFECT OF CORTICOTROPIN AND ADRENAL STEROIDS ON THE MANAGEMENT OF ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

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*General considerations.* Corticotropin and the adrenal steroids have been used widely and with a considerable degree of success in the management of acute disseminated lupus erythematosus (L.E.).<sup>1, 2, 3, 4, 5, 6, 7, 8</sup> The present study is concerned with the use of these agents in the treatment of 55 patients with this illness. This study was originally begun in 1949 and has continued to date. All patients were admitted to the ward service of the hospital for the initial studies and control of the acute manifestations of the disease, this hospital stay varying from four weeks to nine months. The patients were subsequently discharged to a special outpatient department, where they continued to be seen by the same group of investigators who participated in the study at the time they were in the hospital. In this way, continuity and control could be maintained for prolonged periods.

In TABLE 1 are listed the age, sex, and race distribution of the 55 patients. Although acute disseminated lupus erythematosus is a disease predominantly of the female, it does occur not infrequently in the male. Approximately 17 per cent of our group of patients are males, and this percentage corresponds quite well to the 15 to 20 per cent incidence in the male reported in the literature. Early reports described this illness as occurring almost exclusively in the female. It is difficult to know whether the recent increase in the occurrence of the disease in males represents an actual increase in incidence or an improvement in diagnostic acumen provided by the demonstration of the L.E. cell. In general, the male patient is less likely to show the classical florid acute manifestations so often encountered in the female. The diagnosis in the former is, therefore, very often dependent on the degree of clinical suspicion exercised by the physician and the demonstration of the presence of the L.E. cell in the peripheral blood. Of our nine male patients, only two showed the florid form of the disease, including the typical facial butterfly eruption. In the remaining seven males, the illness was more subtle in its manifestations, although equally widespread. All showed the presence of the L.E. cell in the peripheral blood.

Acute disseminated lupus erythematosus may occur at any age from childhood to old age. Our youngest patient is 6 years old and our oldest is a man of 70. The disease most frequently occurs, however, between the ages of 16 and 32. The Negro is not immune to this illness, and in our series it has been observed in both male and female. The general clinical manifestations in the Negro are indistinguishable from those seen in the white patients. There are two families in our series in which the disease has occurred in two siblings of each. Such a familial incidence of the illness has been described before.<sup>9</sup> It is interesting to note that the clinical manifestations of the disease in the



TABLE 1  
AGE, SEX, AND RACE DISTRIBUTION OF 55 PATIENTS WITH ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

	No. of patients
Sex	
Male	9
Female	46
Age in years	
0-10	2
11-20	13
21-30	18
31-40	10
41-50	4
51-60	6
61-70	2
Race	
White	46
Negro	6
Other*	3

\* Mexican, Puerto Rican.

paired siblings may vary considerably. In the first group of siblings that we encountered, sisters aged 11 and 13 years, the 11-year-old child developed a fulminating acute disseminated lupus erythematosus which was associated with early severe renal failure and death several weeks after the onset of the illness. In the 13-year-old sister, the essential manifestations were those of recurrent moderate fever, arthralgias, and a mild facial eruption. This child has been ill for several years, is well controlled by therapy, and has developed no renal impairment. In the other sibling couple, a sister and brother in their middle 20's in age, the sister has had episodes of considerable fever, a facial rash, and evidence of renal involvement. In the brother, the major manifestations are those of moderate joint pains with occasional mild arthritis.

*Clinical considerations.* The patients in our series had evidence of the disease for a variable period of time before they first received corticotropin or adrenal steroid therapy. This pretreatment period of overt disease varied from 4½ weeks to 15 years. It is of some importance to note that 8 of the 55 patients had active recurrent disseminated lupus erythematosus for more than 5 years before more specific therapy was instituted (TABLE 2). This point becomes important in attempting to evaluate the efficacy of any therapeutic measure in this illness. It cannot be emphasized too strongly that not all instances of disseminated lupus erythematosus are of the acutely florid type with an urgent and evil prognosis.

In TABLES 3, 4, and 5 are listed the various signs and symptoms which occurred at one time or another during the course of the illness. In almost half the patients, joint pains were the initial manifestation of the disease while, in almost all patients, such arthralgias occurred at some time or other during the course of the illness and, in half of the group, the arthralgias were associated with definite physical changes in one or more joints. Fever, which occurred with a frequency almost as great as that of arthralgia, rarely manifested itself

TABLE 2

DURATION OF ILLNESS PRIOR TO TREATMENT WITH CORTICOTROPIN OR CORTISONE IN 55 PATIENTS WITH ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

Years	No. of patients
0- $\frac{1}{2}$	16
$\frac{1}{2}$ -1	16
1-2	6
2-3	2
3-4	5
4-5	2
6	1
7	2
9	1
10	1
13	1
14	1
15	1

TABLE 3

INITIAL SYMPTOM OR SIGN WITH ONSET OF ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS IN 55 PATIENTS

Symptoms	No. of patients
Arthralgia.....	24
Rash.....	11
Arthritis.....	8
Fever.....	3
Hoarseness and lymphadenopathy.....	1
Nonspecific weakness.....	1
Alopecia.....	1
Pleuritic pain.....	1
Weight loss.....	1
Abdominal pain.....	1
Convulsions.....	1
Bleeding tendency (purpura).....	1
Edema.....	1

early in the course of the disease. Such pyrexia varied from mildly febrile episodes to instances in which the temperature exceeded 105° F. An eruption was observed to occur at some time or another in two thirds of our group, while it was evident as the initial manifestation in only 20 per cent of the patients. The rash generally assumed the typical butterfly pattern over the bridge of the nose and the malar eminences, but in several instances was confined entirely to the upper extremities and chest. The characteristic shallow ulcerated mucous membrane lesions, particularly of the oral cavity, were seen in one third of the group. Enlargement of the liver occurred in one half the patients and a definite splenomegaly in a quarter. Some cardiovascular or pulmonary abnormalities were observed in one third of the patients. When hypertension was present before treatment, it was invariably associated with significant renal disease. A diffuse lymphadenopathy was noted in 71 per cent of the patients. Such adenopathy generally involved the posterior cer-

TABLE 4

INCIDENCE OF SYMPTOMS IN 55 PATIENTS WITH ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

Symptom	Patients	
	Number	Per cent
Arthralgia.....	52	95
Fever.....	50	91
Weight loss.....	36	65
Rash.....	34	62
Arthritis.....	34	62
Chest pain.....	20	36
Chills or chilliness.....	17	31
Abdominal pain.....	11	20
Convulsions.....	11	20
Alopecia.....	10	18
Lymphadenopathy.....	9	16
Light sensitivity.....	6	11
Bleeding tendency.....	6	11
Paresthesia.....	1	2

TABLE 5

INCIDENCE OF PHYSICAL SIGNS IN 55 PATIENTS WITH ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

Physical sign	Patient	
	Number	Per cent
Lymphadenopathy.....	39	71
Rash.....	34	62
Mucous membrane lesions.....	19	35
Joint abnormalities.....	28	51
Hepatomegaly.....	26	47
Cardiac abnormalities (total).....	20	36
Hypertension.....	7	13
Significant murmur.....	6	11
Gallop rhythm.....	5	9
Pericardial friction rub.....	4	7
Pericardial effusion.....	4	7
Pulmonary abnormalities (total).....	18	33
Pleural effusion.....	7	13
Friction rub.....	6	11
Splenomegaly.....	14	25
Psychiatric abnormalities.....	13	24
Edema.....	12	22
Finger-tip skin lesions.....	9	16
Fundal abnormalities.....	9	16
Petechiae.....	6	11
Neurological abnormalities.....	5	9

vical chain, and the axillary and inguinal nodes. The glands were usually discrete, nontender, and rubbery in consistency. Convulsions occurred in one fifth of the patients *before* treatment was instituted.

The presence of a normal or reduced total white blood cell count is the usual finding in patients with acute disseminated lupus erythematosus. Thirty-

TABLE 6

## HEMATOLOGIC DATA IN 55 PATIENTS WITH ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

	No. of patients
White blood count, cells/cu. mm.	
1000- 3000	8
3100- 5000	27
5100- 9000	15
9100-13000	5
Nonsegmented polys, greater than 10%	33
Hemoglobin, grams/100 cc.	
5.0- 7.0	9
7.1- 9.0	14
9.1-11.0	17
11.1-13.0	15
Coombs' test	
Number of patients	26
Positive Coombs' test	8
Clinical hemolytic syndrome	4
Thrombocytopenia (platelets less than 100,000/cu.mm.)	9

five of our 55 patients had a definite leukopenia and, in only 5 instances, did the white blood cell count vary from 9,000 to 13,000 per cu.mm (TABLE 6). In one third of the group there was a definite shift to the left of the polymorphonuclear leukocytes. The presence of a significant leukocytosis for which there is no adequate explanation should raise suspicion concerning the accuracy of the diagnosis of acute disseminated lupus erythematosus. Some degree of anemia is almost always present, and in 23 members of our group the hemoglobin varied from 5.0 to 9.0 grams. The anemia is usually of the normochromic, normocytic type, and unresponsive to iron therapy. A positive Coombs' test was present in eight of our patients, and four of this group had a definite clinical hemolytic syndrome. Nine individuals in the series showed a moderately severe thrombocytopenia. Six of these nine patients had bleeding tendencies with recurrent crops of petechiae. A splenectomy was performed in one of the patients and, following operation, the platelet count promptly returned to normal levels and the hemorrhagic tendency subsided.<sup>8</sup>

Approximately half the patients showed some renal function abnormalities, as evidenced by the presence of red blood cells in the urinary sediment and varying degrees of albuminuria. In slightly over one third of the group, the blood urea nitrogen was elevated (TABLE 7). Renal function studies, such as urine concentration tests, phenolsulphonphthalein excretion, and urea and creatinine clearances, were determined in slightly more than half the patients (TABLE 8). Several points become evident as a result of these determinations: (1) the urine concentration test proved to be the least sensitive of the renal function tests as observed in this group; (2) the 15-minute excretion of phenolsulphonphthalein and the urea clearance yielded the most useful information in terms of the appraisal of the renal status in our group of patients; (3) evidence of significant impairment in renal function was almost invariably encountered in those individuals who succumbed to the disease. It is our opinion that the renal status, after prolonged and adequate therapy, is the single



TABLE 7

LABORATORY DATA IN 55 PATIENTS WITH ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

	Patients	
	Number	Per cent
Positive L. E. test.....	55	100
Elevated sedimentation rate.....	51	93
Hyperglobulinemia.....	39	71
Cephalin flocculation test (2+ to 4+)*.....	30	68
WBC less than 5000/cu. mm.....	35	64
Urine		
Sediment, occasional to many WBC.....	32	58
Sediment, occasional to many RBC.....	22	40
Sediment, occasional to many casts.....	14	25
Albumin, 1+ or more.....	27	49
Hemoglobin less than 10 gm./100 cc.....	29	53
Abnormal EEG**.....	8	50
Abnormal chest X ray.....	20	36
Azotemia.....	17	31
Creatinine greater than 1.5 mg./100 cc.†.....	12	41
False positive serologic test for syphilis.....	15	27
Abnormal EEG.....	12	22
Prolonged bleeding time‡.....	4	22
Platelets less than 100,000/cu. mm.....	9	16
C-reactive protein test, 1+ or more††.....	8	67
Differential sheep cell agglutinin test, titer greater than 1:16§.....	1	14

\* 44 patients.

\*\* 16 patients.

† 29 patients.

‡ 18 patients.

†† 12 patients.

§ 7 patients.

TABLE 8

KIDNEY FUNCTION STUDIES IN PATIENTS WITH ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

	Patients studied		Per cent		
		Number	Mean	Maximum	Minimum
Phenolsulphonphthalein excretion in 15 min.	Living	14	27	60	3
	Died	3	17	20	12
Phenolsulphonphthalein excretion in 2 hours	Living	20	65	95	35
	Died	3	50	60	35
Urea clearance, cc./min.	Living	4	55	76	40
	Died	5	27	39	10
Creatinine clearance, cc./min.	Living	5	66	82	42
	Died	—	—	—	—
Urinary concentration test	Living	21	1.026	1.032	1.020
	Died	4	1.026	1.030	1.020

most important factor in determining the prognosis of the individual patient. Evidence of persistent or progressive impairment of kidney function is in general an ominous sign.

The characteristic L.E. cell was found in the peripheral blood of all the patients in our series. The blood sedimentation rate was increased in almost the entire group, and a hyperglobulinemia was present in slightly less than three quarters of the patients. In two thirds of the group, there was evidence of some impairment of liver function, as indicated by the cephalin flocculation test. False positive serologic tests for syphilis were encountered in more than one fourth of the patients. The C-reactive protein was not infrequently present, and of considerable interest is the fact that, despite the frequency with which joint manifestations simulating rheumatoid arthritis occurs in acute disseminated lupus erythematosus, the differential sheep cell agglutinin titer was greater than 1:16 in only 1 of 7 patients tested.

*Effects of therapy.* All patients were admitted to the hospital ward service for the institution of therapy. They were placed on a 200 mgm. (8.5 mEq.) sodium diet obtained from the diet kitchen, and weight and blood pressure determinations were recorded daily. Two to 6 gm. of potassium chloride was routinely administered, orally, to all the patients in the group, to prevent the possible development of hypokalemia and hypochloremic alkalosis. Antibiotic therapy was used where indicated, and mercurial diuretics and digitalis were employed in those instances where congestive failure occurred.

The initial daily dose of corticotropin varied from 100 to 150 mgm., given intramuscularly in four divided doses. In the more acutely ill patients, the corticotropin was administered intravenously, 20 to 40 mgm. being dissolved in 500 to 1,000 cc. of 5 per cent glucose in distilled water and given slowly over an 8-hour period. In five patients, zinc corticotropin\* was the initial form of therapy employed (CHART 1). This agent was given intramuscularly in amounts of 20 to 40 mgm. in 2 divided doses. The response to this corticotropin was prompt and gratifying. When cortisone was employed, the initial daily amount generally varied from 200 to 400 mgm., but occasionally as much as 800 mgm. was necessary to induce a remission. The steroid was usually administered by mouth in 4 divided doses, around the clock. One patient with acute disseminated lupus erythematosus was treated for a short period of time with daily oral administration of 9 to 12 mgm. alpha-9-fluorohydrocortisone† (CHART 2). When the acute manifestations of the disease were brought under control, the daily dosage of the agent employed was gradually reduced until the minimal amount consistent with optimal control of the disease was reached. The average daily maintenance dose of cortisone was found to vary from 50 to 100 mgm., although an occasional patient did quite well on 25 mgm. The daily maintenance dosage of corticotropin varied from 25 to 75 mgm. In 13 of the 55 patients, treatment could be entirely discontinued for variable periods of time.

Of our group of 55 patients, 38 are alive and 17 have died. Of the 17 patients

\* Supplied by Doctor Kenneth W. Thompson, of Organon, Inc., Orange, N. J.

† Supplied by Doctor Elmer Alpert of Merck and Co. Inc., Rahway, N. J.

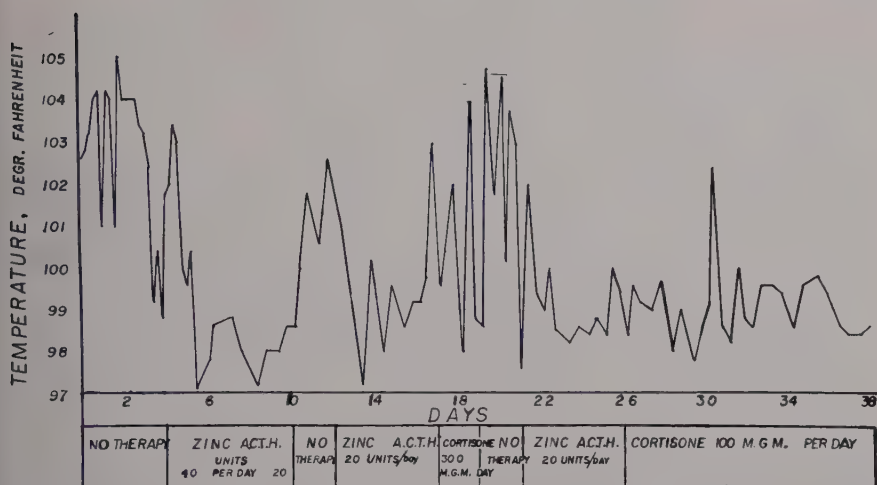


CHART 1. Response of fever to zinc ACTH.

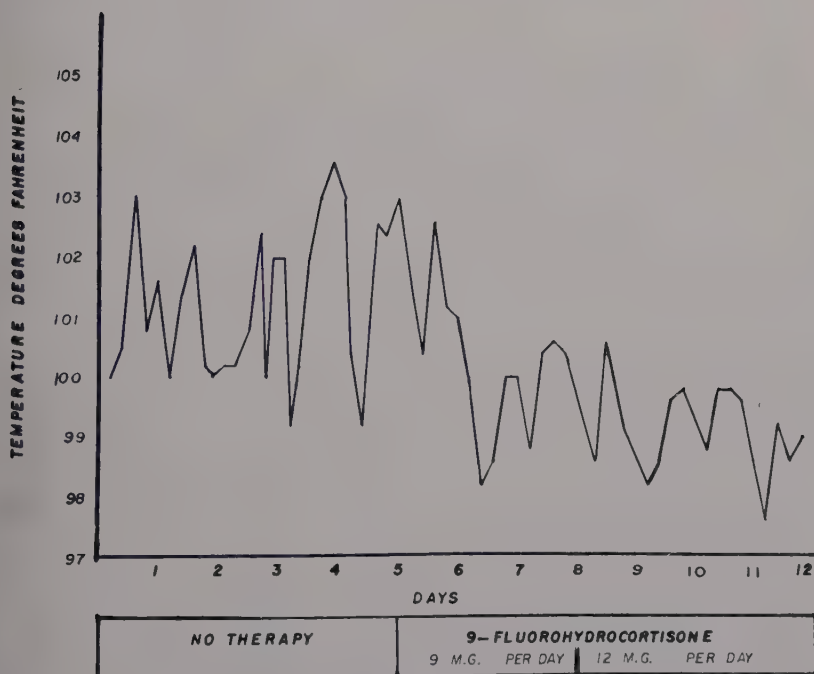


CHART 2. Response of fever to 9-fluorohydrocortisone.

who died, 13 succumbed to progressive and intractable renal failure, one patient committed suicide, one died in status epilepticus which occurred 8 days after the beginning of treatment; one patient succumbed to a disseminated fungus infection, and one died during the early days of therapy when only small and inadequate amounts of the drugs were available. Of the 38 patients who are alive, 13 are in a state of remission during which they have received no form of therapy for 2, 3, 5, 6, 6, 6, 6, 8, 8, 12, 17, 28, and 30 months, respectively. The remaining patients require the daily oral administration of cortisone to prevent recrudescence of the illness. The longest period of continuous treatment to date is 60 months. Of the 38 surviving patients, 26 have a marked and continued clinical improvement and, in 13, the improvement is more modest in character, with frequent, although mild, joint pains and occasional febrile episodes.

In TABLE 9 are listed the effects of therapy on the various clinical manifestations of the disease. There are certain evidences of the illness which respond more readily to treatment than others. Thus, in all patients, the fever and arthralgias disappeared, either entirely or almost so. The rash and mucous membrane lesions subsided, the lymphadenopathy generally tended to become less pronounced, and two thirds of the patients showed significant gains in body weight. Similarly, pleural effusions and friction rubs, and to

TABLE 9  
EFFECT OF TREATMENT WITH CORTICOTROPIN OR CORTISONE ON THE CLINICAL  
MANIFESTATIONS OF ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

Clinical manifestation	Total No. of patients	Completely improved	Partially improved	Total improved
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Fever.....	47	62	38	100
Arthralgia.....	50	50	50	100
Weight loss.....	35	66	—	66
Lymphadenopathy.....	36	19	36	56
Rash.....	32	41	50	91
Mucous membrane lesions.....	19	53	21	74
Hepatomegaly.....	25	8	12	20
Cardiac abnormalities.....	20	25	10	35
Hypertension.....	7	0	0	0
Gallop rhythm.....	5	20	40	60
Pericardial friction rub.....	4	50	0	50
Pericardial effusion.....	4	50	0	50
Significant murmur.....	6	0	0	0
Pulmonary abnormalities.....	18	72	22	94
Pleural friction rub.....	6	83	17	100
Pleural effusion.....	7	71	0	71
Chest pain.....	20	55	35	90
Psychiatric abnormalities.....	13	31	31	62
Finger-tip skin lesions.....	9	11	33	44
Abdominal pain.....	11	36	36	73
Edema.....	12	25	33	58
Splenomegaly.....	14	7	7	14
Alopecia.....	9	33	11	44
Fundal abnormalities.....	9	44	22	67
Neurological abnormalities.....	5	0	20	20



TABLE 10

EFFECT OF TREATMENT WITH CORTICOTROPIN OR CORTISONE ON THE LABORATORY DATA IN ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

Laboratory test	Number of patients before therapy	Percentage of patients improved
Positive L.E. test.....	55	0
Elevated ESR.....	49	57
Hyperglobulinemia.....	37	46
WBC less than 500/cu. mm.....	32	72
Hemoglobin less than 10 gm./100 cc.....	28	54
Urine		
Sediment, occasional to many RBC.....	22	32
Sediment, occasional to many WBC.....	32	50
Sediment, occasional to many casts.....	14	36
Albumin, 1 plus or more.....	27	26
Abnormal chest X ray.....	20	60
False positive serologic test for syphilis.....	14	29
Azotemia.....	17	35
Abnormal EEG.....	8	0
Shift to left (nonsegmented forms greater than 10%).....	32	41
Reticulocytes greater than 0.7%.....	19	37
Cephalin flocculation greater than 1 plus.....	29	14
Abnormal electrocardiogram.....	12	42
Prolonged bleeding time.....	4	75
C-reactive protein test greater than 1 plus.....	8	25
Platelets less than 100,000.....	8	63
Positive direct Coombs' test.....	8	0
Hemolysis.....	4	100

a somewhat lesser extent pericardial effusions, were absorbed. Previously existent hypertension, however, remained unaffected. It is important to emphasize again that the pretreatment presence of hypertension generally indicates the existence of underlying renal disease. In only a small number of patients did there occur a significant improvement in renal function, as evidenced by a decrease in albuminuria and in the number of red blood cells found in the urinary sediment and a reduction in the blood urea nitrogen (TABLE 10). In some patients, the presence of an increase in the blood urea nitrogen before treatment is instituted is the result of fever and dehydration in the absence of true renal disease. In this group, general improvement resulting from therapy is followed by a reduction in or disappearance of the azotemia. Neither the hepatomegaly nor the splenomegaly is particularly affected by therapy, while the psychiatric abnormalities were improved in 8 of 13 patients.

It is of considerable interest that, in no instance, did the L.E. cell entirely disappear from the peripheral blood, regardless of the degree of remission induced by treatment (TABLE 10). During the period of marked improvement, the L.E. cells are decreased appreciably in number and may, indeed, be very difficult to find, but a careful search of the peripheral blood will reveal their presence at some time or another. The sedimentation rate fell to normal levels in over half the cases, and there occurred a reduction in the hyper-

globulinemia in almost half the group. There occurred an increase in the peripheral white blood cells, and, in half the patients, some improvement in the anemia. The thrombocytopenia responded well in five of eight patients, and the hemolytic process disappeared in the four patients in whom it was present prior to treatment. In no instance, however, did a positive Coombs' test become negative.

In attempting to evaluate the over-all results of therapy it becomes evident that acute disseminated lupus erythematosus is not cured either with corticotropin or with the adrenal steroids. There are many manifestations of the illness which are either not at all or only minimally affected by these measures. The lack of any appreciable influence on the development and progression of renal disease plays a most important role in determining the prognosis of the individual patient. Many other acute and chronic manifestations of the disease, however, are promptly brought under control with these agents. A significant percentage of the patients are satisfactorily rehabilitated and, with continuous or intermittent therapy, are maintained in a reasonably good state of health. These hormonal fractions, therefore, constitute a very significant advance in the management of this illness. On the basis of our limited experience, alpha-9-fluorohydrocortisone proved to be 10 to 15 times as potent as cortisone, milligram for milligram, in control of the acute manifestations of the disease. This fraction, however, also shows a marked increase in its ability to cause a retention of salt and water, and its use will, therefore, be limited by this property. Zn ACTH exercised a very satisfactory effect on the acute phase of the illness. Twenty to 40 units, given in 1 or 2 daily subcutaneous or intramuscular injections, proved entirely adequate for control of the acute manifestations of the disease.

*Complications of therapy.* In TABLE 11 are presented comparisons between

TABLE 11  
COMPARISON OF INCIDENCE OF COMPLICATIONS DURING TREATMENT WITH  
CORTICOTROPIN OR CORTISONE ENCOUNTERED IN FIRST 32  
PATIENTS AND LAST 23 PATIENTS

Manifestation	Early series (32 patients)	Later series (23 patients)
	<i>per cent</i>	<i>per cent</i>
Rounding of face.....	75	18
Psychiatric abnormalities.....	63	9
Hypertension.....	56	13
Edema.....	50	0
Infections.....	47	13
Acne.....	40	13
Alopecia.....	31	13
Hirsutism.....	28	13
Convulsions.....	25	9
Congestive heart failure.....	22	0
Metabolic alkalosis.....	19	9
Abdominal striae.....	16	0
Diabetes.....	6	4
Osteoporosis.....	3	9
Peptic ulcer.....	3	0

complications of treatment observed in the first 32 patients of our series and those encountered in the more recent 23 members of the group. The majority of the latter individuals have received treatment for approximately two years. It is of interest to observe the decrease in almost all the side effects of treatment in the more recent group, since we have learned to employ the agents more effectively with regard to both the initial and maintenance dosages.

*Summary.* Fifty-five patients with acute disseminated lupus erythematosus were treated with corticotropin and adrenal steroids. The series included 46 females and 9 males, ranging in age from 6 to 70 years.

The members of the group had evidence of the disease for periods varying from  $4\frac{1}{2}$  weeks to 15 years before treatment with these fractions was instituted.

Thirty-eight of the 55 patients are alive and 17 have died. Thirteen of the deaths were due to progressive renal failure.

Of the 38 living patients, 13 are in a state of remission during which they have received no form of therapy for 2 to 30 months. The longest period of continuous therapy to date in our group is 60 months.

The clinical manifestations of the disease, the laboratory findings, and the influence of therapy on these data is described.

The side effects observed with treatment are outlined and the more recent decrease in the incidence of such side effects is discussed.

The agents employed in the treatment of the disease in our series include corticotropin, Zn corticotropin, cortisone, and, in one instance, alpha-9-fluorohydrocortisone.

### References

1. BAEHR, G. & L. J. SOFFER. 1950. Treatment of disseminated lupus erythematosus with cortisone and adrenocorticotropin. *Bull. N. Y. Acad. Med.* **26**: 229.
2. CAREY, R. A., A. M. HARVEY & J. E. HOWARD. 1950. The effect of adrenocorticotrophic hormone (ACTH) and cortisone on the course of disseminated lupus erythematosus and periarteritis nodosa. *Bull. Johns Hopkins Hosp.* **87**: 425.
3. SOFFER, L. J., M. F. LEVITT & G. BAEHR. 1950. Use of cortisone and adrenocorticotrophic hormone in acute disseminated lupus erythematosus. *Arch. Internal Med.* **86**: 558.
4. SOFFER, L. J., G. BAEHR, M. F. LEVITT & M. BADER. 1951. The use of adrenocorticotropin and cortisone in acute disseminated lupus erythematosus. *Proc. 2nd Clin. ACTH Conf.* **2**: 680. J. R. Mote, Ed. Blakiston. Philadelphia, Pa.
5. BRUNSTING, L. A., C. H. SLOCUMB & J. W. DIDCOCK. 1951. Effects of cortisone on acute disseminated lupus erythematosus. *Arch. Dermatol. and Syphilol.* **63**: 29.
6. DUBOIS, E. L., R. R. COMMONS, P. STARR, C. S. STEIN, JR. & R. MORRISON. 1952. Corticotropin and cortisone treatment for systemic lupus erythematosus. *J. Am. Med. Assoc.* **149**: 995.
7. SOFFER, L. J. & R. BADER. 1952. Corticotropin and cortisone in acute disseminated lupus erythematosus. Results of long-term use. *J. Am. Med. Assoc.* **149**: 1002.
8. SOFFER, L. J., S. K. ELSTER & D. J. HAMERMAN. 1954. Treatment of acute disseminated lupus erythematosus with corticotropin and cortisone. *Arch. Internal Med.* **93**: 503.
9. DAVIS, M. W. & G. H. GUTRIDGE. 1951. Disseminated lupus erythematosus in identical twin sisters, associated with diabetes mellitus in one case. *J. Missouri State Med. Assoc.* **48**: 446.

# THE USE OF CORTICOTROPIN, CORTISONE, AND HYDROCORTISONE IN NEPHROSIS OF CHILDHOOD

By Conrad M. Riley

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Since the introduction of corticotropin and cortisone into clinical medicine in 1949, it has become apparent that one could induce diuresis in edematous patients with childhood nephrosis in about three out of four tries. Since no immediate change occurs with treatment that can be measured by any available technique, and since one has to wait 10 to 14 days to gauge its effectiveness, it has been impossible accurately to titrate the optimal dose and the optimal length of time to give the hormones. On an empirical basis we have adopted, the general practice of giving a large dose (100 mg. ACTH or 200 mg. of cortisone daily to a two- three-year-old child) for a period of 10 to 14 days.

The comparison of one drug with another has been difficult. As the number of cases increases, however, we have developed an impression that there is no appreciable difference between the over-all effects of corticotropin and cortisone. In pediatrics, we have usually preferred cortisone because it can be given by mouth. Sometimes, however, after a cortisone failure, ACTH proves successful. This can be explained, we think, on the postulated failure of absorption of the orally administered drug in a patient with an edematous gut. We have not made the critical experiment of comparing intramuscular cortisone with intramuscular ACTH.

Our experience with hydrocortisone is limited to very small numbers—six to eight cases. In this brief trial, we have seen no obvious difference from either of the other hormones.

Of much more concern than the effectiveness of the individual drugs is whether any of these agents significantly alters the basic disease. In the past few weeks, several doctors (TABLE 1) have submitted answers to questionnaires\* about their patients under 12 years of age. With these pooled figures, we have been able to collect information which appears to have statistical significance.

Our criteria for the diagnosis of "nephrosis" have been very broad and we have accepted all patients with anasarca, massive proteinuria, hypoproteinemia, and hypercholesterolemia regardless of whether they might show signs of "chronic glomerulonephritis." We have asked the persons reporting only if the patients were alive or dead and whether they received no adrenocortical-active hormone therapy, such therapy for edema only, or such therapy on a preplanned schedule.

This is a preliminary report of the first 533 answers. Since questionnaires are still being returned, we shall be able to report more fully later.

It rapidly became apparent in our own group that, with the introduction

\* This study was supported by the National Nephrosis Foundation, Inc., New York, N. Y.



TABLE 1

SOURCES OF CASES USED IN EVALUATING ADRENOCORTICAL-ACTIVE HORMONE THERAPY IN CHILDHOOD NEPHROSIS

Reporting Physician	Location	Number of Cases
Doctor Clark West	Cincinnati	37
Doctor Conrad Riley	New York City	112
Doctor A. J. Merrill	Atlanta	10
Doctor W. Wm. McCrory	Philadelphia	58
Doctor M. Frances McCall	Montreal	40
Doctor Henry Barnett	New York City	94
Doctor Kurt Lange	New York City	44
Doctor Walter Heymann	Cleveland	97
Doctor Lawrence Greenman	Pittsburgh	41
		533

of treatment, there was no significant change in the proportion of patients dying to those going on to "complete cure" with clearing of chemical abnormalities as well as clinical improvement. Therefore, we decided to make a study of length of life after the onset of the disease instead of using "cure" as an end point.

First, we considered those cases with no hormone therapy of this type. Second, we considered those where treatment had been given sporadically for edema only. The difference in survival time of these two was insignificant. Thus, for comparison purposes, we felt justified in pooling them.

Next, we turned to patients whose treatment was planned ahead of time without regard to edema. The administration of hormone in such "preplanned

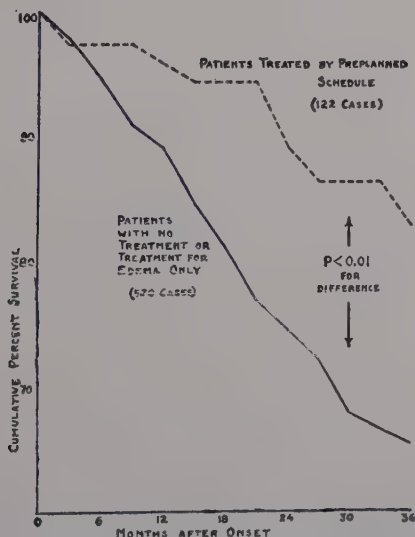


FIGURE 1. Survival rate of cases of childhood nephrosis with and without preplanned adrenocortical-active hormone therapy.

schedules" varied from 3 days out of each week, to 10 days out of each month, to 28 days continuously, to indefinite continuous treatment. In this group of 122 cases (FIGURE 1), had there been deaths at the same rate as in the first mentioned two groups, there would have been 18.2 deaths after  $2\frac{1}{2}$  years (30 months). The actual observed deaths were 6. This difference, when tested statistically, might have occurred by chance in less than 1 in 100 times. This statement must still be qualified by the fact that our statistical advisors feel that the numbers both of the control group and the treated group are not as large as they would like. Also the fact that the cases compared were not necessarily contemporary must make us remember that this is still a preliminary report. But it is encouraging.

If we accept the above figures as suggesting a truly favorable influence of preplanned hormone therapy, then what is the best type of therapy? This question cannot be answered absolutely, because no one program has been given an adequate trial. I should merely like to report the system that we have been using for the past five months in our clinic. This system, so far, looks satisfactory.

We found that the erythrocyte sedimentation rate, which is invariably very high in the edematous state, falls consistently on hormone treatment if the patient does well clinically. At the time of diuresis, however, it may not have fallen to normal. Therefore, we have made a policy of continuing treatment until the sedimentation rate does reach normal—sometimes up to four weeks. After this, we repeat the test at one- to two-week intervals. If the sedimentation rate begins to climb above normal, we again begin treatment. If it has risen only a little, it falls, with treatment, to normal in a week or so. In our short experience, it seems that if we can keep this measurement normal or close to normal, the child will remain clinically well and, often, has little or no protein in the urine. Further and more extensive trials of this approach are obviously necessary.

In summary, we have presented statistical evidence which suggests that adrenocortical-active hormone therapy, though it may not produce real cures, at least significantly prolongs life, if used in some form of preplanned schedule. Though no incontrovertible evidence is available to indicate what is the best type of "preplanned schedule," it is our impression that maintaining the erythrocyte sedimentation rate at or near normal is a practicable and useful guide in treatment.

### Part III. Selected Parenteral Forms of Hydrocortisone in Therapy

#### A. INTRAVENOUS USE

#### PHARMACOLOGICAL STUDIES IN MAN OF 11-, 17-, AND 21-HYDROXY DERIVATIVES OF PROGESTERONE AND THEIR FLUORINATED ANALOGS\*

By Alan Goldfien, William I. Morse, E. Rudolf Froesch, W. Francis Ganong, Albert E. Renold, and George W. Thorn

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The oxygenated derivatives of progesterone include such highly active compounds as hydrocortisone and corticosterone, whose biological effects in experimental animals and in man have been extensively studied. Other compounds in this series, however, have been available in small amounts only, and studies of their activity in man are few.<sup>1</sup>

In view of the remarkable potentiation of hydrocortisone by the substitution of a fluorine atom at the 9 $\alpha$  position,<sup>2, 3, 4, 5, 6</sup> it seemed desirable to explore the metabolic activity of other 9 $\alpha$  fluorinated steroids. Samples of the 9 $\alpha$  fluorine derivatives of hydrocortisone, corticosterone, 11 $\beta$ -hydroxyprogesterone and 11, 17-dihydroxyprogesterone have been obtained (FIGURE 1); for purposes of comparison, the studies were extended to include the nonfluorinated analogs and 11 $\alpha$ -hydroxyprogesterone. The intravenous route of administration was chosen as providing the most accurate basis for comparison of the small quantities of hormone available.

*Methods.* These studies were carried out in the Metabolic Unit of the Peter Bent Brigham Hospital. Patients with proved Addison's disease were allowed to come into balance on a constant diet before studies began. Sufficient time was allowed for them to return to equilibrium between the successive administration of the compounds being studied. The urinary sodium and potassium values were determined by flame photometry. The urinary glucose was estimated by the method of Renold and Froesch,<sup>7</sup> in which the urinary reducing substances are compared before and after incubation with glucose oxidase. Other determinations were carried out as previously described.<sup>8</sup> For intravenous administration the compounds were dissolved in ethanol and added to either 5 per cent dextrose in water or saline. On control days, similar infusions were given without addition of steroids.

*Observations.* The effects of 25 mg. of progesterone, 11 $\alpha$ -hydroxyprogesterone, 11 $\beta$ -hydroxyprogesterone and 12 mg. of 9 $\alpha$ -fluoro-11 $\beta$ -hydroxyprogesterone on urinary sodium, potassium and glucose excretion were studied in a 20-year-old male with Addison's disease. The steroids were dissolved in 20 ml. of ethanol, which was added to 250 cc. of 5 per cent dextrose and water.

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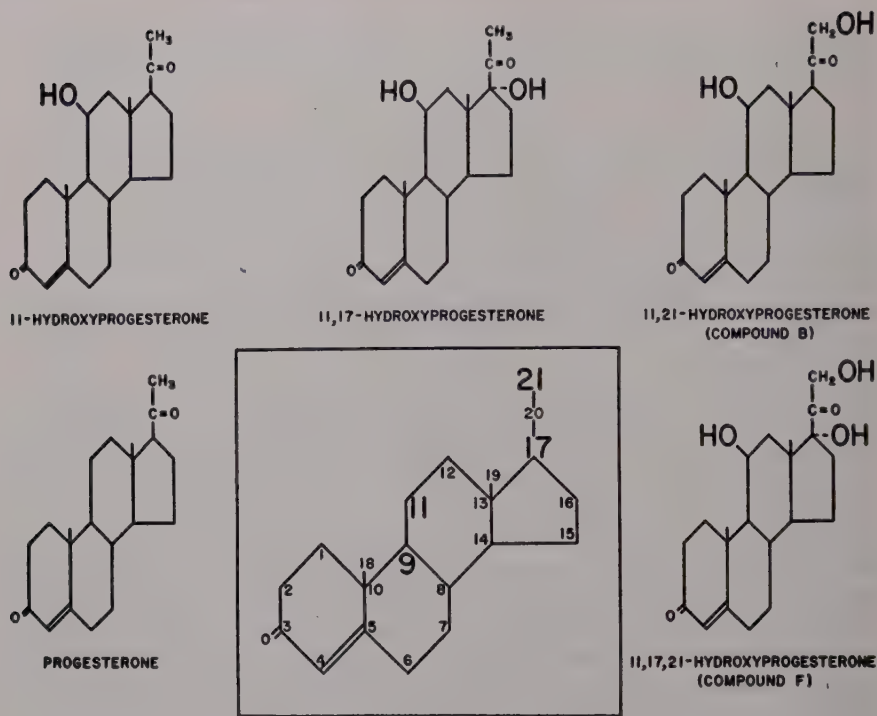


FIGURE 1

This solution was administered intravenously over 30 minutes. In this instance, maintenance therapy was omitted during the control periods reported, as well as on the days the test substances were being given. Upon reaching equilibrium, the patient was excreting 167 mEq. of sodium and 70 mEq. of potassium per 24 hours in the urine. The results of this study are illustrated in FIGURE 2, the first column representing the control day on which the patient was given an infusion of dextrose and water with ethanol. Progesterone itself induced minimal sodium retention whereas 11 $\alpha$ -hydroxyprogesterone, at the same dose level (25 mg.) showed no effect. 11 $\beta$ -Hydroxyprogesterone produced a sodium retention equal to that of progesterone. This was associated with a small increase in glucose excretion. At the 12 mg. dose level, the 9 $\alpha$ -fluoro-11 $\beta$ -hydroxyprogesterone produced sodium retention, increased potassium excretion, and an increase in urinary glucose. In only the latter instance did the effects exceed those of the maintenance dose (0.5 mg.) of 9 $\alpha$ -fluorohydrocortisone.

In the second study, the effects of 45 mg. of 11,17-dihydroxyprogesterone and 25 mg. of the 9 $\alpha$ -fluorinated 11,17-dihydroxyprogesterone were compared in a 48-year-old male with Addison's disease maintained on a constant diet and 0.5 mg. of fluorohydrocortisone daily by mouth. In this instance, the steroids were infused intravenously over an eight-hour period. Maintenance



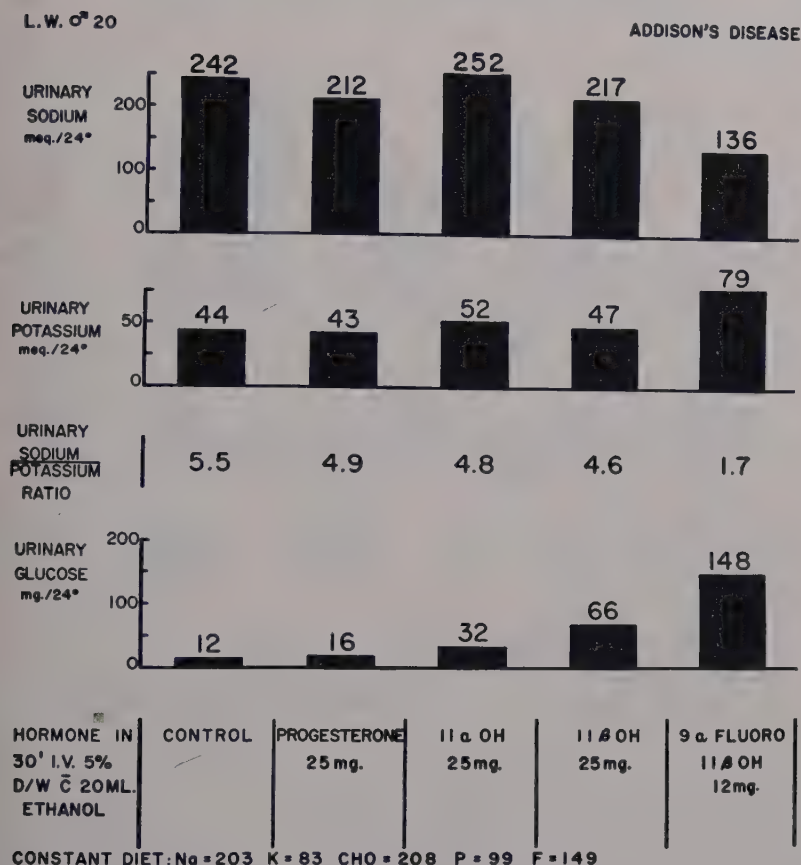


FIGURE 2. The effects of 25 mg. of progesterone, 11 $\alpha$ -hydroxyprogesterone, 11 $\beta$ -hydroxyprogesterone and of 12 mg. of 9 $\alpha$ -fluoro-11 $\beta$ -hydroxyprogesterone intravenously administered to a patient with Addison's disease.

therapy was omitted on the control and test days. The results of this study are illustrated in FIGURE 3. In the first column are the results of the dextrose-water-ethanol infusion without added steroids. The administration of 11,17-dihydroxyprogesterone resulted in a sodium diuresis, a small and probably not significant increase in glucose excretion and an eosinophil fall of 45 per cent in 8 hours. The fluorinated analog, however, produced a marked sodium retention, potassium, and glucose diuresis, as well as a 67 per cent fall in eosinophils in 8 hours.

The effects of 25 mg. of the 9 $\alpha$ -fluorinated derivatives of 11 $\beta$ -hydroxyprogesterone, 11,17-dihydroxyprogesterone and hydrocortisone on urinary sodium, potassium, and glucose excretion of a 38-year-old male with Addison's disease maintained in balance on a constant diet and 25 mg. of cortisone are illustrated in FIGURE 4. Cortisone was omitted on the control and test days. The compounds were administered intravenously over 8 hours in 500 ml. of normal

H. McC. ♂ 48

ADDISON'S DISEASE

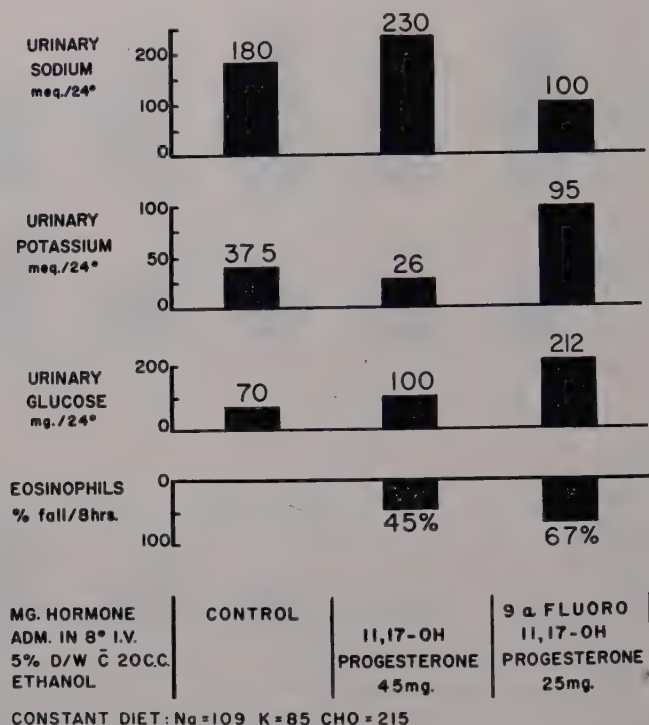


FIGURE 3. The effect of 45 mg. of 11,17-dihydroxyprogesterone and of 25 mg. of 9α-fluoro-11,17-dihydroxyprogesterone in a 38-year-old male with Addison's disease.

saline solution, with 20 ml. of ethanol and 5 grams of albumin. It can be seen that all three substances produced sodium retention and an increase in potassium and glucose excretion. Although 9α-fluoro-11β-hydroxyprogesterone produced a greater sodium and potassium effect than 9α-fluoro-11,17-dihydroxyprogesterone, the latter compound produced a significantly greater increase in glucose excretion. The marked increase in glucose excretion with the fluorohydrocortisone is noteworthy.

In the fourth study, the effects of 1 mg. of the 9α-fluorohydrocortisone were compared to those of 1 mg. of 9α-fluorocorticosterone. Both substances were administered over 8 hours in an intravenous infusion of 500 ml. of 5 per cent dextrose and water to a 46-year-old patient with Addison's disease. The changes in blood eosinophils and urinary sodium and potassium are illustrated in FIGURE 5. Although 9α-fluorohydrocortisone produced a greater potassium loss and eosinopenia, 9α-fluorocorticosterone produced the greater sodium retention. When 50 mg. of nonfluorinated corticosterone were administered to this patient under identical conditions, the 24-hour urinary sodium was 86 mEq.; the urinary potassium 88 mEq. No eosinopenic effect or increase in glucose excretion was noted.

H.W. ♂ 38

## ADDISON'S DISEASE

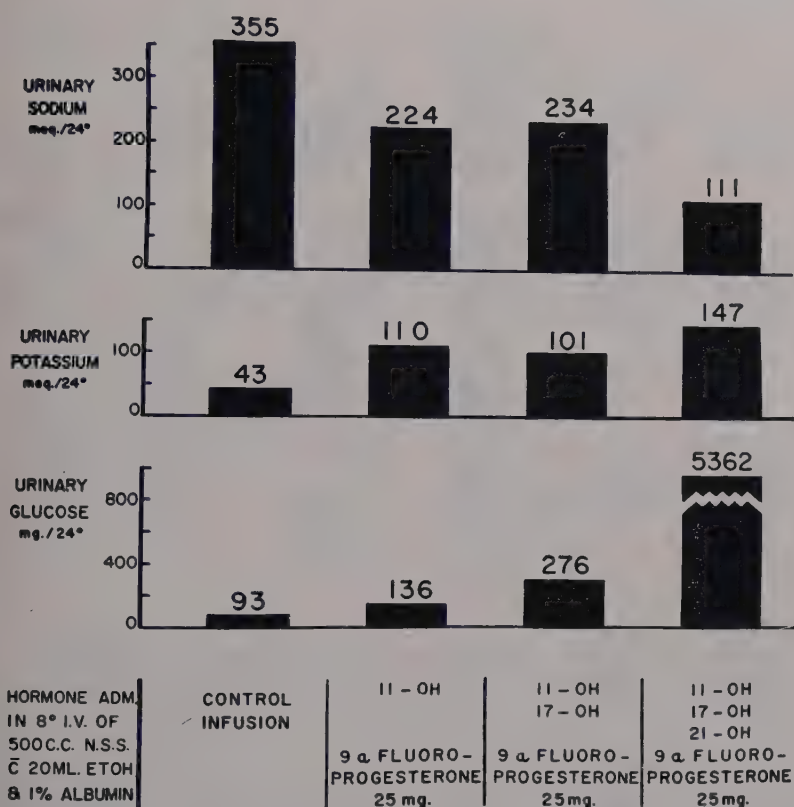
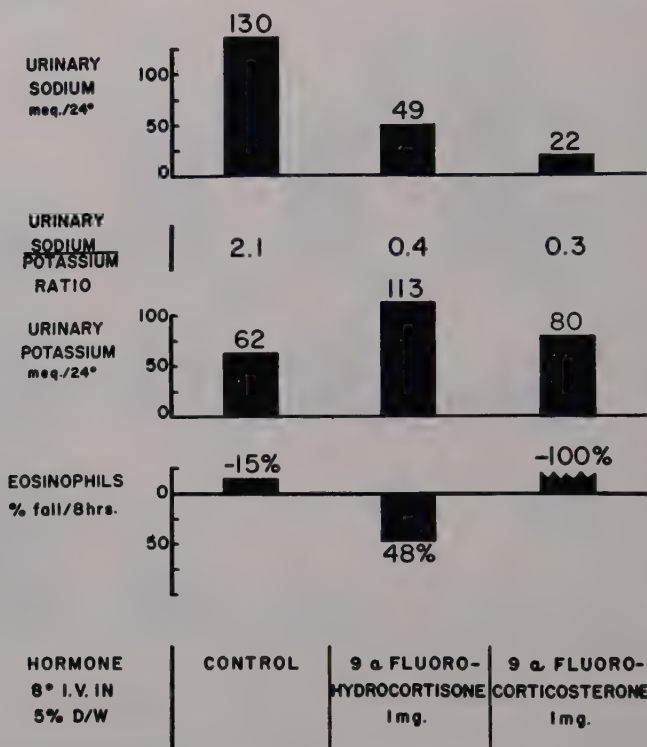


FIGURE 4. The effect of 25 mg. of the 9 $\alpha$ -fluorine derivatives of 11 $\beta$ -hydroxyprogesterone, 11,17-dihydroxyprogesterone and hydrocortisone administered intravenously to a 38-year-old male with Addison's disease.

Before 9 $\alpha$ -fluorocorticosterone became available as the free alcohol for intravenous studies, 50 mg. of the 9 $\alpha$ -fluorine derivatives of 11-hydroxy- and 11,17-dihydroxyprogesterone were compared to 5 mg. of the acetates of fluorocorticosterone and fluorohydrocortisone by mouth in a 50-year-old woman with Addison's disease maintained on a constant diet and 1.25 mg. of desoxycorticosterone daily by intramuscular injection. The results are illustrated in FIGURE 6. 9 $\alpha$ -Fluoro-11 $\beta$ -hydroxyprogesterone produced sodium retention, potassium diuresis and a small increase in glucose excretion. When compared to this compound, 9 $\alpha$ -fluoro-11,17-dihydroxyprogesterone was again noted to exert a greater eosinopenic and glucosuric action with a less marked sodium-retaining effect at this dose level. At one-tenth the dose level, 9 $\alpha$ -fluorocorticosterone and 9 $\alpha$ -fluorohydrocortisone displayed considerably greater activity in sodium retention and potassium diuresis. A comparison of the latter two compounds showed that for similar electrolyte effect the glucosuric

W.L. ♂ 46

ADDISON'S DISEASE



CONSTANT DIET: Na = 115 K = 85

FIGURE 5. The effects of 1 mg. of 9α-fluorohydrocortisone and 1 mg. of 9α-fluorocorticosterone administered intravenously to a 46-year-old male with Addison's disease.

and eosinopenic effect of the fluorohydrocortisone was considerably more marked.

*Discussion.* The results of the present study confirm previous experience with fluorohydrocortisone in that the substitution of a fluorine atom at the 9α position in 11-oxygenated steroids results in marked enhancement of metabolic activity.<sup>2, 3, 4, 5</sup> The data obtained with the compounds studied were analyzed by pairing results obtained in the same individual so as to relate the addition of a hydroxyl group at the 11α, 11β, 17- or 21 positions of progesterone to a change in metabolic activity (TABLE 1). The addition of the 11α-hydroxyl group decreased the salt-retaining effect of progesterone. The 11β-hydroxyl function did not alter the sodium-retaining activity when added to progesterone. In the presence of the 21-hydroxyl group, however, a marked reduction of sodium-retaining activity was noted. This was associated with an increase in glucocorticoid activity. The 11β-hydroxyl function appears to be necessary for glucocorticoid activity. The main metabolic effect of the addition of a 17-hydroxyl group appeared to be an increase in glucocorticoid



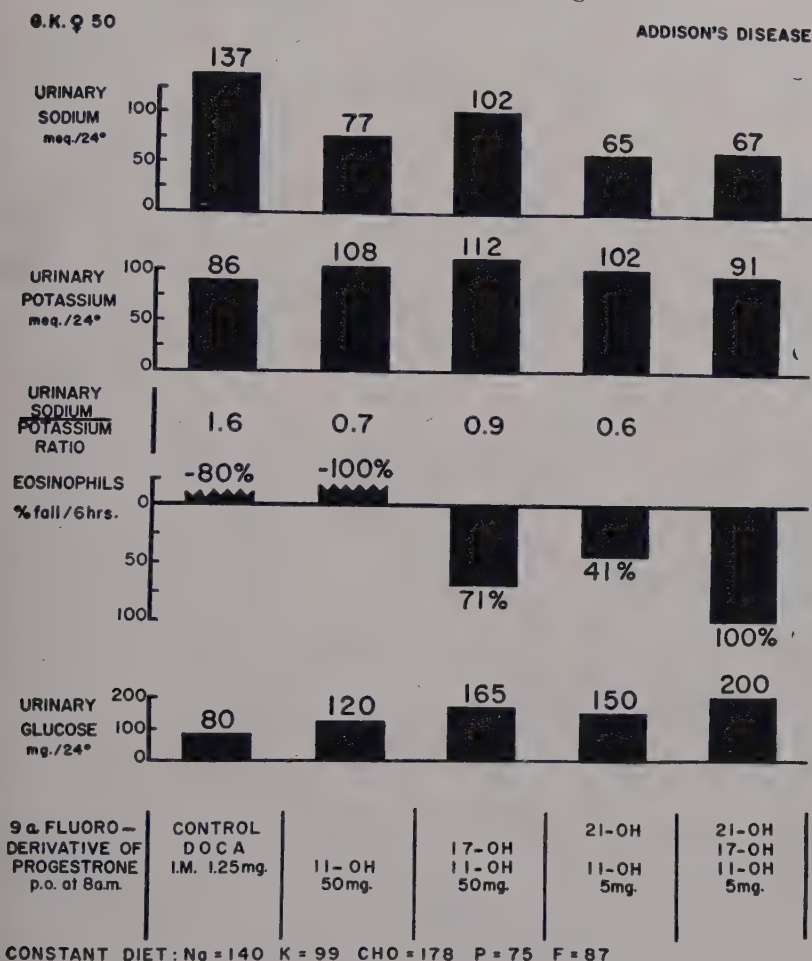


FIGURE 6. The effects of 50 mg. of 9 $\alpha$ -fluoro-11 $\beta$ -hydroxyprogesterone, 50 mg. of 9 $\alpha$ -fluoro-11,17-dihydroxyprogesterone, 5 mg. of 9 $\alpha$ -fluorocorticosterone and 5 mg. of 9 $\alpha$ -fluorohydrocortisone administered as a single oral dose to a 57-year-old female with Addison's disease maintained on 1.25 mg. of 11-desoxycorticosterone in oil by daily intramuscular injection.

activity. In previous studies it had been shown, however, that the addition of the 17-hydroxyl group in the absence of the 11 $\beta$ -hydroxyl group (11-desoxycorticosterone versus compound S (17,21-dihydroxyprogesterone) led to a marked reduction in the sodium-retaining action without appreciable enhancement of the glucocorticoid effect.<sup>9</sup> When a 17-hydroxyl group is added to progesterone the resulting compound has significant androgenic activity. The addition of a 21-hydroxyl group primarily enhances sodium-retaining activity. That it may also enhance glucocorticoid activity (to a lesser degree) is illustrated by the greater degree of glucocorticoid activity of hydrocortisone as compared with that of 11,17-dihydroxyprogesterone.

TABLE 1  
A COMPARISON OF THE METABOLIC EFFECT OF OXYGENATED DERIVATIVES OF  
PROGESTERONE

Compound	Change in structure	Principal metabolic change
Progesterone		
11 $\alpha$ -Hydroxyprogesterone	+11 $\alpha$ OH	None
Progesterone		
11 $\beta$ -Hydroxyprogesterone	+11 $\beta$ OH	$\uparrow$ CHO $\pm$
Desoxycorticosterone*		
Corticosterone*	+11 $\beta$ OH	$\downarrow$ Mineral
11 $\beta$ -Hydroxyprogesterone		
11 $\beta$ , 17 $\alpha$ -Dihydroxyprogesterone	+17 $\alpha$ OH	$\uparrow$ CHO
Corticosterone		
Hydrocortisone	+17 $\alpha$ OH	$\uparrow$ CHO
11 $\beta$ -Hydroxyprogesterone		
Corticosterone	+21 OH	$\uparrow$ Mineral
11 $\beta$ , 17 $\alpha$ -Dihydroxyprogesterone		
Hydrocortisone	+21 OH	$\uparrow$ Mineral
Progesterone*		
Desoxycorticosterone	+21 OH	$\uparrow$ Mineral
9 $\alpha$ -Fluoro-11 $\beta$ -hydroxyprogesterone		
9 $\alpha$ -Fluoro-11 $\beta$ , 17 $\alpha$ -dihydroxyprogesterone	+17 $\alpha$ OH	$\uparrow$ CHO
9 $\alpha$ -Fluorocorticosterone		
9 $\alpha$ -Fluorohydrocortisone	+17 $\alpha$ OH	$\uparrow$ CHO
9 $\alpha$ -Fluoro-11 $\beta$ -hydroxyprogesterone		
9 $\alpha$ -Fluorocorticosterone	+21 OH	$\uparrow$ Mineral
9 $\alpha$ -Fluoro-11 $\beta$ , 17 $\alpha$ -dihydroxyprogesterone		
9 $\alpha$ -Fluorohydrocortisone	+21 OH	$\uparrow$ Mineral

\* Except for these compounds, the above comparisons were made on data obtained from the same individual.

Although the 9 $\alpha$ -fluorine-substituted compounds showed greatly enhanced activity, the relationship of these substances to each other did not appear to be significantly altered (TABLE 1). The data reported by Fried in animal studies support this conclusion.<sup>10</sup> It was of interest to note that in compounds of low biological activity, such as 11 $\beta$ -hydroxyprogesterone and 11,17-dihydroxyprogesterone, the substitution of a fluorine atom at the 9 $\alpha$  position greatly facilitated their study, since smaller quantities produced measurable effects, thus obviating difficulties in obtaining large quantities of the compounds and of preparing them for intravenous administration.

To date 9 $\alpha$ -fluorohydrocortisone has been shown to be useful as a diagnostic tool and therapeutic agent.<sup>5, 6</sup> Further studies are in progress to determine the possible usefulness of the other compounds.

*Summary.* Studies of the effects of 11 $\alpha$ -hydroxyprogesterone, 11 $\beta$ -hydroxy-

progesterone, 11,17-dihydroxyprogesterone, hydrocortisone, and corticosterone, and of the 9 $\alpha$ -fluorine-substituted analogs of the latter four compounds, confirm earlier observations made with fluorohydrocortisone that the 9 $\alpha$ -fluorine substitution results in marked enhancement of metabolic activity. By correlating the metabolic effects of these compounds with the hydroxyl substitutions in the positions 11, 17, and 21 of progesterone, the following conclusions were supported: (1) the 11 $\beta$ -hydroxyl group appeared to be necessary for glucocorticoid activity, but in the presence of the 21-hydroxyl group led to decreased sodium-retaining activity; (2) the 17 $\alpha$ -hydroxyl group increased glucocorticoid activity; (3) the 21-hydroxyl group primarily enhanced sodium-retaining activity. Other effects were noted and briefly discussed. The conclusions here reported are to be considered preliminary.

### References

1. REICHSTEIN, T. & C. W. SHOPPEE. 1943. The hormones of the adrenal cortex. *Vitamins and Hormones*. Harris and Thimann, Ed. **1**: 345-413.
2. FRIED, J. & E. R. SABO. 1954. 9 $\alpha$ -Fluoro derivatives of cortisone and hydrocortisone. *J. Am. Chem. Soc.* **76**: 1455.
3. BORMANN, A., F. M. SINGER & P. NUMEROF. 1954. Growth-survival and sodium retaining activity of 9 $\alpha$ -halo derivatives of hydrocortisone. *Proc. Soc. Exptl. Biol. Med.* **86**: 570-573.
4. LIDDLE, G. W., M. M. PECHET & F. C. BARTTER. 1954. Enhancement of biological activities of corticosteroids by substitution of halogen atoms in 9 $\alpha$  position. *Science*. **120**: 496-497.
5. GOLDFIEN, A., J. C. LAIDLAW, N. ABU HAYDAR, A. E. RENOLD & G. W. THORN. 1955. Fluorohydrocortisone and chlorohydrocortisone, highly potent derivatives of compound F. *New Engl. J. Med.* **252**: 115-421.
6. RENOLD, A. E., N. ABU HAYDAR, W. J. REDDY, A. GOLDFIEN, J. R. ST. MARC & J. C. LAIDLAW. 1955. Biological effects of fluorinated derivatives of hydrocortisone and progesterone in man. *Ann. N. Y. Acad. Sci.* **61**(2): 582-590.
7. RENOLD, A. E. & E. R. FROESCH. In preparation.
8. FORSHAM, P. H., G. W. THORN, F. T. G. PRUNTY & A. G. HILLS. 1948. Clinical studies with pituitary adrenocorticotropin. *J. Clin. Endocrinol.* **8**: 15-66.
9. THORN, G. W., L. L. ENGEL & R. A. LEWIS. 1941. The effect of 17-hydroxycorticosterone and related adrenal cortical steroids on sodium and chloride excretion. *Science*. **94**: 348-349.
10. FRIED, J. 1955. Biological effects of 9-alpha-hydrocortisone and related halogenated steroids in animals. *Ann. N. Y. Acad. Sci.* **61**(2): 573-581.

# THE EFFECT OF INTRAVENOUSLY ADMINISTERED HYDROCORTISONE ON THE URINARY 17-KETOSTEROIDS IN PATIENTS WITH ADRENAL VIRILISM\*

By Joseph W. Jailer and Eleanor Z. Wallace

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The dramatic decrease in urinary 17-ketosteroids (17-KS) which occurs as a result of the prolonged intramuscular administration of cortisone in patients with congenital adrenal hyperplasia was first shown by Wilkins and his collaborators (1951), and has been amply confirmed (Bartter, Albright, *et al.*, 1951; Jailer, 1951). Hydrocortisone has been shown to be equally effective (Jailer, Louchart, and Cahill, 1952). When these steroids are given intramuscularly, four to five days may elapse before the 17-KS fall to values which are considered approximately normal for the age and sex of the patient. The oral route of administration of the steroids, on the other hand, is not as effective in suppressing the adrenal cortical secretion; consequently, approximately twice the dosage of steroid must be given to achieve a comparable fall in the urinary 17-KS.

The mechanism by which cortisone or hydrocortisone exert its effects is presumably by ACTH inhibition and secondary adrenocortical atrophy accompanied by decreased hormonal secretion. That this mechanism is responsible for the fall in urinary 17-KS can be best illustrated by the experiments of Bartter, Albright, and their associates (1951), who have shown that if ACTH and cortisone are given simultaneously, the usual fall in the urinary 17-KS obtained with cortisone alone does not occur. The inhibition of ACTH secretion by these steroids not only occurs in congenital adrenal hyperplasia, but may be found in normal individuals as well, since prolonged administration of cortisone may result in adrenal atrophy and even evidence of adrenal insufficiency (Salassa *et al.*, 1953; Perera and Ragan, 1950).

On the other hand, Jailer, Gold, and Cahill (1952); Gardner and Migeon (1951); Jailer, Gold, and Wallace (1954); and others have shown that, when the adrenal virilism is caused by an adrenal tumor, no significant fall in the urinary 17-KS occurs as a result of the cortisone administration. These observations have led to the hypothesis that adrenal neoplasia differs from hyperplasia in that the former is independent of ACTH control and can maintain the steroid output, even when endogenous ACTH secretion is inhibited.

Since the "cortisone test" as described above may require almost 10 consecutive days of urine collection, it was hoped that, with the availability of a soluble hydrocortisone preparation suitable for intravenous administration, the test could be shortened to a 24- or 48-hour period. Consequently, the following procedure has been adopted: urines were collected in 4-hour periods for 8 to 24 hours before and 16 to 24 hours after the intravenous administration of 50 to 100 mg. of hydrocortisone in glucose and water; the 17-KS were deter-

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B.L.  
C.A.H.

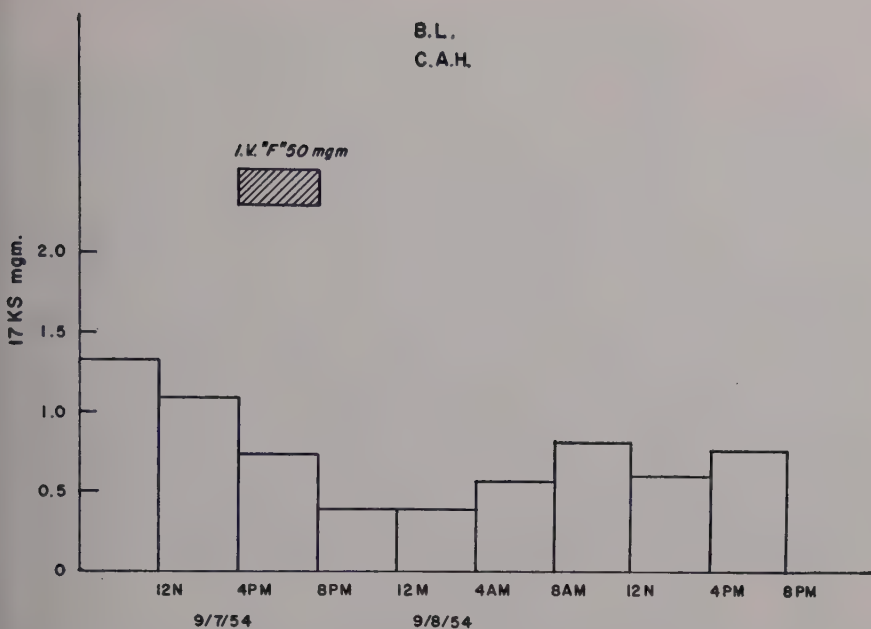


FIGURE 1. B. L., a four-year-old female pseudohermaphrodite with congenital adrenal hyperplasia, to whom 50 mgm. hydrocortisone was given intravenously in 250 ml. of glucose and water.

E.B.  
C.A.H.

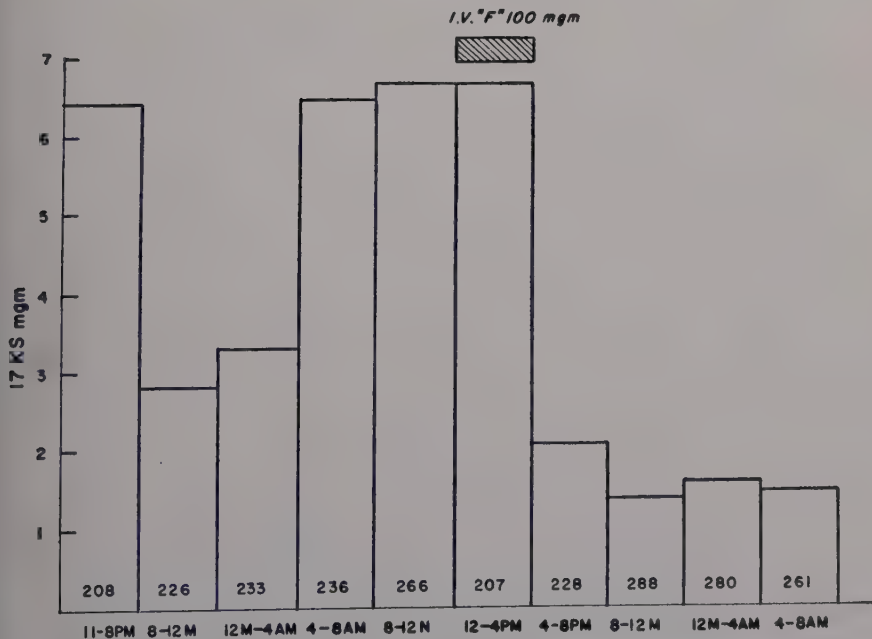


FIGURE 2. E. B., an 11-year-old boy with congenital adrenal hyperplasia, to whom 100 mg. of hydrocortisone was administered in 500 ml. glucose and water.

mined by the Holtorff-Koch modification of the Zimmerman reaction; in a few cases, an indwelling catheter was inserted in the patients to be sure of complete urine collections; creatinine determinations were done on each specimen of urine to check for completeness of the collection. The intravenous hydrocortisone test was employed in three patients with congenital adrenal hyperplasia, in one patient with a benign adrenal adenoma with virilism, and in another patient with an adrenal carcinoma and virilism. In addition to the above, four patients with Cushing's syndrome were also studied in a similar fashion.

**Results.** In the three patients with congenital adrenal hyperplasia, a 4-hour infusion of 50 to 100 mgm. of hydrocortisone caused a marked fall in the urinary 17-KS. In one patient (FIGURE 1), the decrease in urinary steroids occurred during the period of infusion. In the second and third patients, it was observed in the 4-hour period following the completion of the infusion. In all cases, the 17-KS rose again (FIGURES 2 and 3) to preinfusion levels within 12 hours after the end of the infusion.

Two patients with adrenal virilism due to tumor [one with carcinoma (FIG-

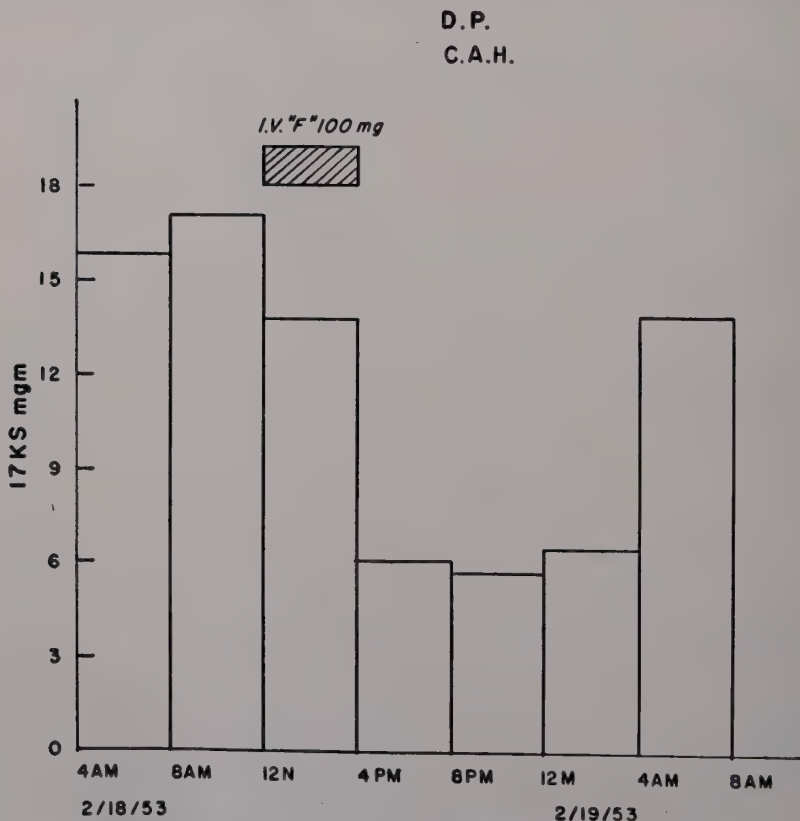


FIGURE 3. D. P., a 17-year-old female with congenital adrenal hyperplasia.

URE 4) and the other with an adrenal adenoma (FIGURE 5)] had no fall in urinary 17-KS excretion with the hydrocortisone infusion.

Four patients with Cushing's syndrome (three with hyperplasia and one with an adrenal adenoma) were also studied. In these patients, the values for 17-KS during the control periods fluctuated widely enough so that doubt could be cast on the significance of any fall obtained.

*Discussion.* When hydrocortisone was administered intravenously to patients with congenital adrenal hyperplasia, a rapid fall in the urinary 17-KS resulted. Where adrenal virilism was caused by an adrenal tumor, no such fall could be induced. Intravenous hydrocortisone appears to be an effective agent for applying the "cortisone test" rapidly in order to differentiate between the two etiologies of the adrenogenital syndrome. Because of the marked fluctuations in 17-KS output which may occur from one 4-hour period to another, however, adequate control periods must be obtained. In some instances, fluctuations may be great enough to hinder interpretation of the fall in 17-KS which occurs. For this reason, the intravenous test cannot be considered accurate enough to replace the more prolonged intramuscular test in adrenal

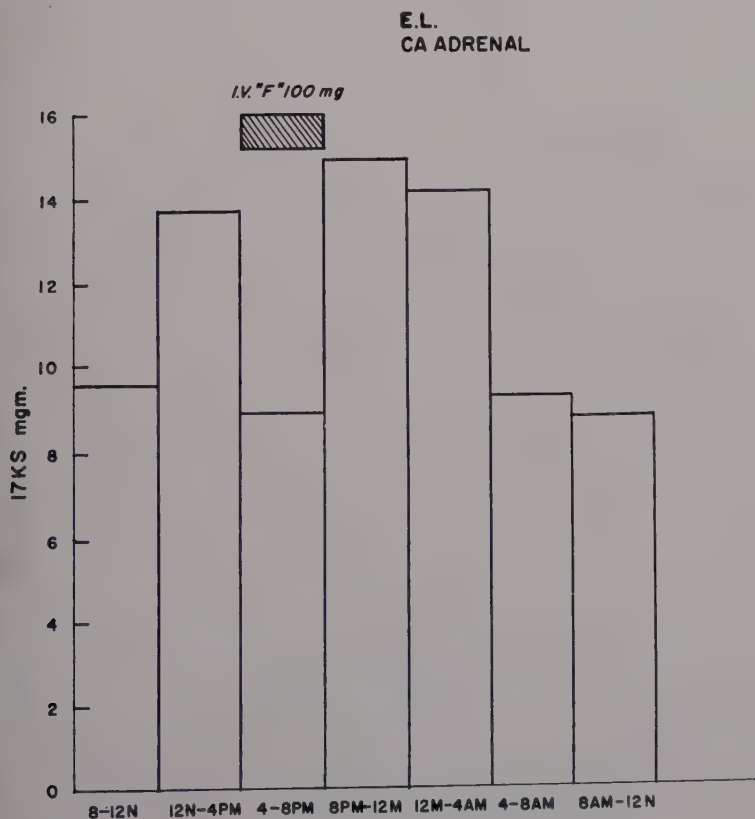


FIGURE 4. E. L., a 21-year-old girl with virilism due to an adrenal carcinoma.

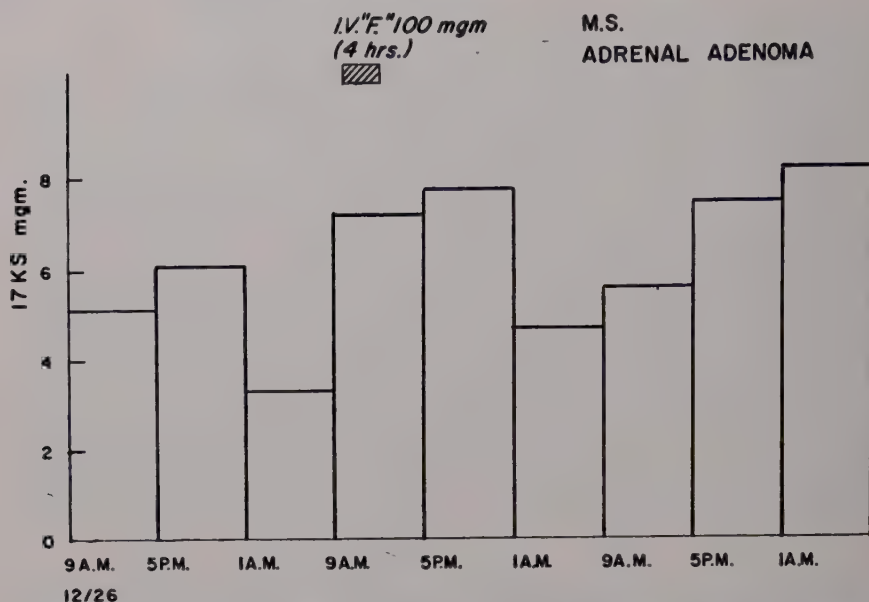


FIGURE 5. M. S., a 33-year-old woman with virilism due to an adrenal adenoma. Urines were collected in eight-hour periods in an attempt to diminish fluctuations from period to period.

virilism. Because the 17-KS in Cushing's syndrome may be normal or low (Forbes and Albright, 1951) and their fluctuations even more marked than in the adrenogenital syndrome, the intravenous hydrocortisone test is extremely difficult to interpret and can be viewed more with interest than for diagnosis.

Physiologically, however, it is of interest that effective plasma levels of hydrocortisone appear able to inhibit ACTH secretion within several hours. Since 17-KS excretion may be suppressed in four hours from the time of hydrocortisone administration, it would appear that there is no significant extra adrenal storage of ketosteroids.

*Summary.* The intravenous administration of 50 to 100 mg. of hydrocortisone over a 4-hour period results in a marked fall in the urinary 17-KS in patients with adrenal virilism when the underlying pathology is congenital adrenal hyperplasia and is without effect when the virilism is due to an adrenal tumor.

### References

- BARTTER, F. C., A. ALBRIGHT, A. P. FORBES, A. LEAF, E. DEMPSEY & E. CARROLL. 1951. The effects of adrenocorticotrophic hormone and cortisone in the adrenogenital syndrome associated with congenital adrenal hyperplasia: an attempt to explain and correct its disordered hormonal pattern. *J. Clin. Invest.* **30**: 237.
- FORBES, A. P. & F. ALBRIGHT. 1951. Comparison of 17-KS excretion in Cushing's syndrome with adrenal tumor and with adrenal hyperplasia. *J. Clin. Endocrinol.* **11**: 926.
- GARDNER, L. I. & C. J. MIGEON. 1951. Le diagnostic des tumeurs virilisantes du cortex surrénalien: effet de la cortisone sur les stéroïdes urinaire et utilisation d'une méthode colorimétrique pour le dosage de la déhydroisoandrosterone. *Helv. Paed. Acta.* **6**: 465.
- JAILER, J. W. 1951. Recent studies on adrenal hyperplasia. *Trans. N. Y. Acad. Sci.* **13**: 262.



- JAILER, J. W., J. LOUCHART & G. F. CAHILL. 1952. Adrenal virilism. I. Diagnostic considerations and treatment. *J. Am. Med. Assoc.* **150**: 575.
- JAILER, J. W., J. J. GOLD & E. Z. WALLACE. 1954. Evaluation of the "cortisone test" as a diagnostic aid in differentiating adrenal hyperplasia from adrenal neoplasia. *Am. J. Med.* **16**: 340.
- PERERA, G. A. & C. RAGAN. 1950. Hypoadrenalism: steroidal mediation of sodium action on blood pressure; modification of antiarthritic response to cortisone. *Proc. Soc. Exptl. Biol. Med.* **75**: 99.
- SALASSA, R. M., W. A. BENNETT, F. R. KEATING, JR. & R. G. SPRAGUE. 1953. Postoperative adrenal cortical insufficiency; occurrence in patients previously treated with cortisone. *J. Am. Med. Assoc.* **152**: 1509.
- WILKINS, L., R. A. LEWIS, R. KLEIN, L. I. GARDNER, J. F. CRIGLER, E. ROSEMBERG & C. J. MIGEON. 1951. Treatment of congenital adrenal hyperplasia with cortisone. *J. Clin. Endocrinol.* **11**: 1.

### *Discussion of the Paper*

DOCTOR HERBERT KUPPERMAN: We have made a comparative study of the ability of various compounds to inhibit the 17-ketosteroids when they are administered orally. An 18-year-old girl with adrenal hyperplasia, whose control 17-ketosteroid excretion was 60 mg. a day, was maintained on a 25 mg. dose of cortisone acetate twice a day for a period of one year with excellent control. She menstruated after the oral administration of cortisone. We found, however, that a total daily dose of 10 mg. of Meticorten was capable of suppressing the 17-ketosteroid excretion to a level equivalent to about 50 mg. cortisone acetate.

Another case was a girl with adrenogenital syndrome who had a control excretion of approximately 40 mg. every 24 hours. When this patient was placed on 12½ mg. of cortisone three times a day, the ketosteroid excretion was suppressed moderately. When we changed it to free hydrocortisone, 10 mg. three times a day, we got further suppression, which we believe is significant in that hydrocortisone was more effective than cortisone. When we reverted to cortisone again, the ketosteroid excretion increased once more. We were able to quantitate the results when, again on Meticorten, 10 mg. total dose a day, the patient showed a marked drop in ketosteroid excretion.

In still another patient with the adrenogenital syndrome, the control excretion was between 60 and 80 mg. per 24 hours. When placed on 5 mg. twice a day of Meticorten, there was a marked drop in the ketosteroids. When we changed to 1 mg. three times a day of 9-alpha-fluorohydrocortisone, the 17-ketosteroids fell even lower.

It is our impression that the technique of following the urinary 17-ketosteroid excretion in these patients can be used as a method of evaluating the comparative activity of various compounds in suppressing ACTH secretion.

DOCTOR JAILER: In our experience, the use of orally administered steroids resulted in a great variation in the values of the 17-ketosteroid excretion from day to day. Consequently, isolated levels were of little or no value in ascertaining the therapeutic effectiveness of any steroid.

## THE USE OF INTRAVENOUS HYDROCORTISONE IN MAJOR SURGERY\*

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### *Introduction*

In man, hydrocortisone (17-hydroxycorticosterone) represents the major component of the corticosteroids secreted by the adrenal cortex into the peripheral blood.<sup>1, 2</sup> Approximately 80 per cent of the corticosteroids found in adrenal venous blood is hydrocortisone (FIGURE 1). Qualitatively, hydrocortisone appears to produce all of the known physiologic and metabolic effects observed with adrenal cortical stimulation,<sup>2</sup> although other corticosteroids present in the adrenal cortical effluent, such as aldosterone, have a much greater effect on electrolyte metabolism. In man, however, aldosterone production by the adrenal cortex does not seem to be increased by ACTH stimulation (Axelrod *et al.*<sup>3</sup>).

The anti-inflammatory or antiphlogistic<sup>4</sup> activity of hydrocortisone is approximately one and one-half times that of cortisone.<sup>5</sup> Work demonstrating the conversion of cortisone to hydrocortisone by various tissues<sup>6</sup> suggests the possibility that cortisone may be biologically active at the cellular level only after such conversion. These observations would appear to make hydrocortisone the therapeutic agent of choice. The high solubility of hydrocortisone as the free alcohol (FIGURE 2)<sup>7</sup> and its greater metabolic effect represent advantages. Hydrocortisone seems to be more rapidly absorbed than cortisone acetate from the gastrointestinal tract and from intramuscular depots. A period of more than 30 minutes, however, is required before maximal blood levels are obtained.<sup>8, 9</sup>

In therapeutic situations, where it is desirable to achieve high blood levels with maximum rapidity, the intravenous route is indicated.<sup>10</sup> Hydrocortisone is now available in a form suitable for intravenous use, as 20 cc. ampules of 100 mg. free hydrocortisone in 50 per cent alcohol, which must be diluted before use. The half-life of hydrocortisone given intravenously, as measured by urinary 17-hydroxycorticoids, is approximately four hours (FIGURE 3). Hellman *et al.*, using intravenous hydrocortisone-4-C<sup>14</sup> in man, demonstrated that, irrespective of the amount given over a 30-minute period, 75 per cent of the material or its breakdown products is demonstrable in urine or stool within 24 hours. There is, thus, a rapid excretion of hydrocortisone which makes the drug relatively harmless if given in excessive amounts, since its presence in the body and the metabolic effects are short-lived.

Side effects are to be expected, however, whenever the period of infusion

\* We are indebted to Merck and Co. Inc., Rahway, N. J., for supplying Cortone Concentrate for intravenous administration as well as for a grant-in-aid, and to the Upjohn Company, Kalamazoo, Mich., for supplying Cortef Concentrate for intravenous administration and for their support of this investigation.

† Formerly Life Insurance Medical Research Fund Fellow 1953-1954.

## THE CHIEF CORTICOSTEROIDS IN MAN

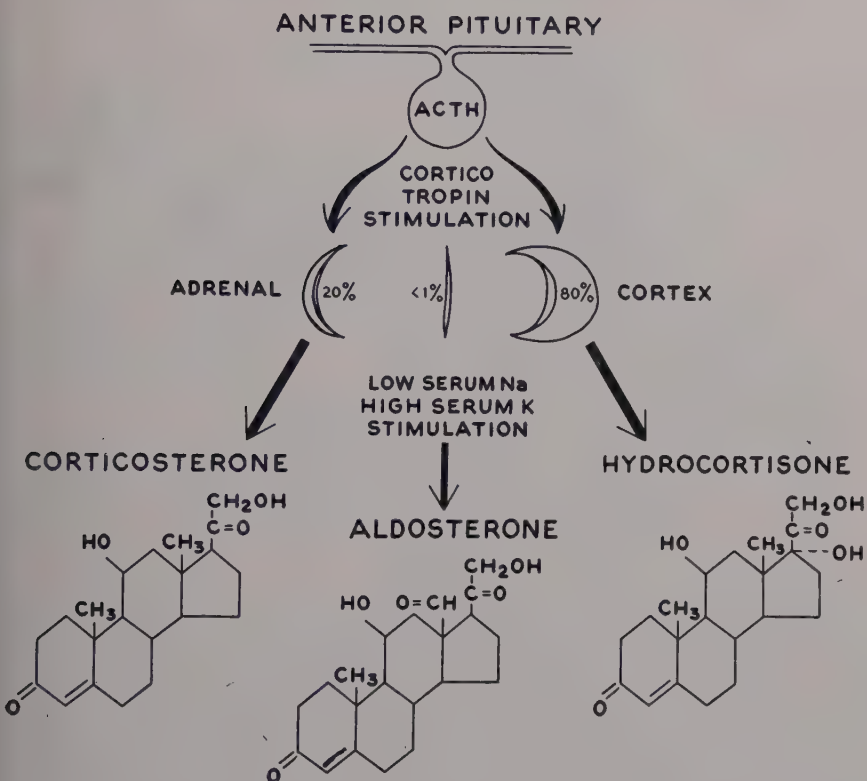


FIGURE 1

SOLUBILITY OF CORTICOIDS  
mg./ml.

Hormone	Water	Plasma	Synovial Fluid
Cortisone	0.28	0.75	0.56
Hydrocortisone	0.28	0.70	0.25
Cortisone Acetate	0.02	0.16	0.36
Hydrocortisone Acetate	0.01	0.02	0.04

FIGURE 2

## URINARY EXCRETION OF INTRAVENOUSLY ADMINISTERED HYDROCORTISONE

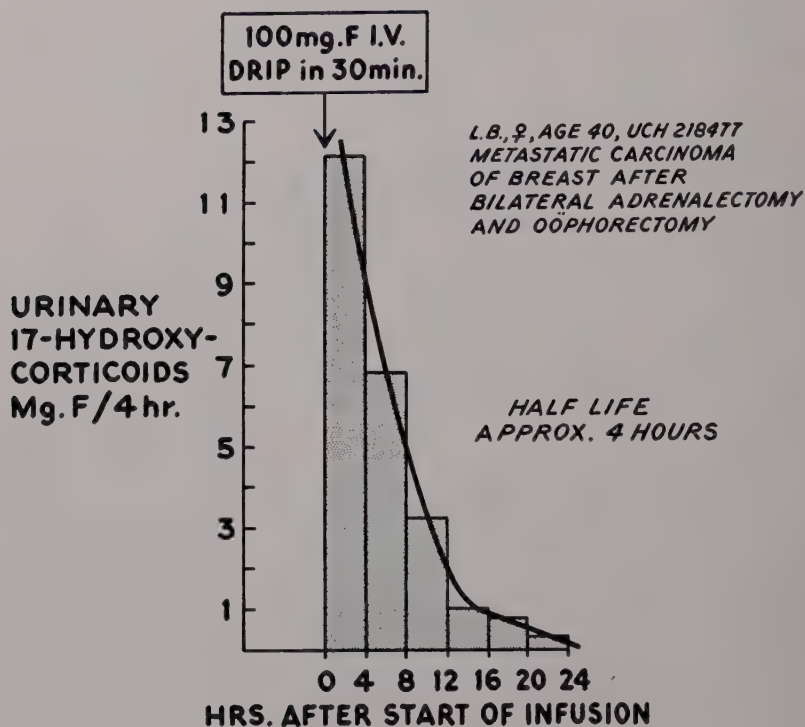


FIGURE 3

is prolonged, or when the rate of inactivation of hydrocortisone is decreased, as in liver disease.<sup>10</sup> Suppression of adrenal cortical activity is another hazard. The intravenous infusion of 100 mg. of hydrocortisone over eight hours will produce transient evidence of adrenal cortical suppression, which is most apparent during the third eight hour period after discontinuing the infusion (FIGURE 4). This phenomenon must be considered even with the temporary use of intravenous hydrocortisone in emergency therapy during surgery and the postoperative period. Longer maintenance therapy and a gradual reduction of the dose may be necessary.

### Observations

Some illustrative examples of the intravenous hydrocortisone during surgery and the postoperative period observed at the University of California Hospital (U.C.H.) are presented:

(1) *Total bilateral adrenalectomy.* During even the most minor surgical procedure requiring anesthesia, the adrenal cortex is normally activated through



COMPARISON OF ADRENAL CORTICAL SUPPRESSION  
PRODUCED BY INTRAVENOUS ADMINISTRATION  
OF HYDROCORTISONE AND 9  $\alpha$  FLUOROHYDROCORTISONE

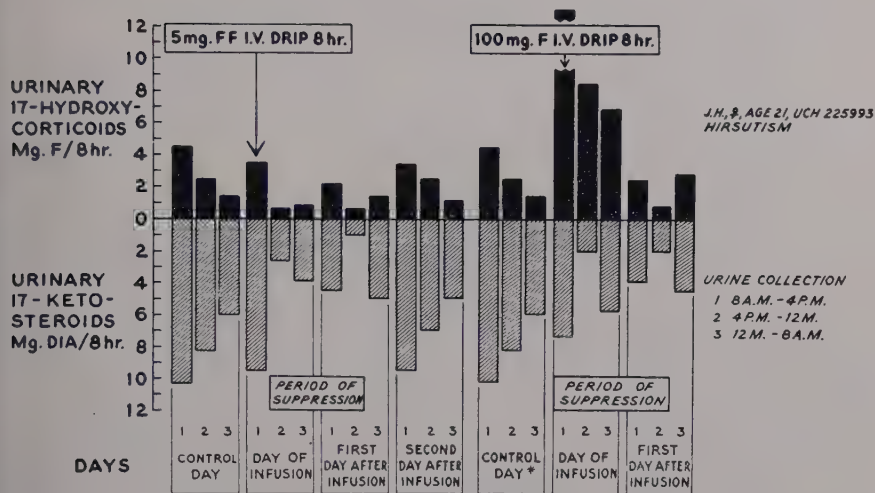


FIGURE 4

hypothalamic-pituitary-adrenocortical stimulation to increase the secretion of steroid hormones.<sup>11</sup> This adrenal response gradually decreases to normal levels postoperatively within five to seven days, as may be demonstrated by measuring the urinary excretion of hydrocortisone and related hormones, designated as 17-hydroxycorticoids (FIGURE 5). With the knowledge that only about 25 to 33 per cent of administered hydrocortisone may be found in the urine, it may be estimated that the output of hydrocortisone by the adrenal cortex during the stress of surgery and anesthesia is approximately 50 to 100 mg. the first day, gradually decreasing to the normal output of 25 to 30

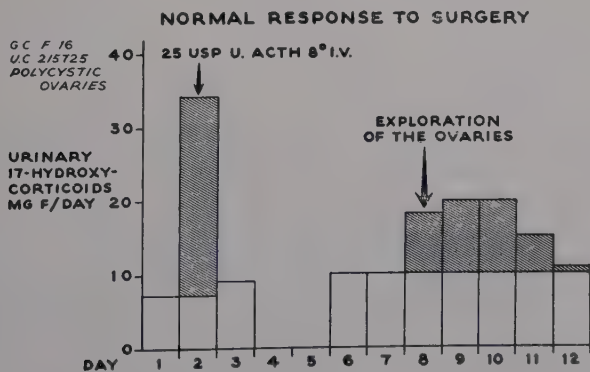
FIGURE 5. From Galante, Rukes, Forsham, and Bell.<sup>17</sup>

TABLE 1  
USE OF INTRAVENOUS HYDROCORTISONE IN MAJOR SURGERY

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PREOPERATIVE (just before induction of anesthesia):	
<i>Infusion No. 1:</i>	100 mg. of hydrocortisone in 1000 ml. of 5 per cent dextrose in water; slow drip (rate according to BP) over 8 hours.
POSTOPERATIVE:	
<i>Infusion No. 2:</i>	100 mg. of hydrocortisone in 1000 ml. of 5 per cent dextrose in saline, 12 to 14 hours.
<i>Infusion No. 3:</i>	50 mg. of hydrocortisone in 1000 ml. of 5 per cent dextrose in water, 12 to 14 hours.
<i>Day 1:</i>	Cortisone acetate, 50 mg. i.m.q. 8 h.
<i>Day 2:</i>	Cortisone acetate, 50 mg. p.o.q. 8 h.
<i>Day 3:</i>	Cortisone acetate, 25 mg. p.o.q. 8 h.
<i>Day 4:</i>	Cortisone acetate, 12.5 mg. p.o.q. 8 h.
<i>Day 5:</i>	Cortisone acetate, 12.5 mg. p.o.q. 12 h.
<i>Day 6:</i>	Steroid therapy discontinued except when maintenance required.

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mg. in about five to seven days. These principles have been used in the management of patients with adrenal insufficiency and hypopituitarism undergoing surgery, and of patients having bilateral adrenalectomies.

Large doses of steroid were given over a period of five days in order to insure adequate adrenal cortical replacement. Directions for hydrocortisone and cortisone administration are given in TABLE 1. There is no special preoperative preparation until the day of operation. Just before the anesthetic induction, an intravenous infusion of 100 mg. of hydrocortisone in 1000 ml. of 5 per cent dextrose-water is started, the rate of infusion was 30 drops per minute, initially, and subsequently regulated as necessary to maintain blood pressure. This infusion was followed by 1000 ml. of 5 per cent dextrose-saline with 100 mg. of hydrocortisone at a slow rate and this injection, in turn, is followed by 1000 ml. of 5 per cent dextrose-water with 50 mg. of hydrocortisone. At least four hours before completion of the intravenous hydrocortisone, the patient was given cortisone acetate intramuscularly or orally, and in gradually decreasing amounts thereafter. Cortisone was discontinued or decreased to maintenance levels by about the sixth postoperative day, unless complications arose. No undesirable side effects have occurred on the dosage scheme outlined. There has been no difficulty with wound healing, and no evidence of adrenal insufficiency. Intravenous infusion of hydrocortisone (as the free alcohol) at rates up to 50 mg. per hour has been carried out in over 50 instances with no untoward effect.

The following case demonstrates that intravenous hydrocortisone will maintain satisfactory blood pressure in patients with adrenal insufficiency undergoing surgery:

*Case of H. K., U.C.H. No. 145337:* This 54-year-old woman with metastatic carcinoma of the breast was admitted in April 1954, for bilateral adrenalectomy and oophorectomy. Thirty minutes before anesthesia induction, a slow intravenous drip of 100 mg. hydrocortisone solution in 5 per cent dextrose and water was started (FIGURE 6). This infusion was continued throughout surgery and immediate postoperative period (total volume 2,000 cc.). The rate of the intravenous drip was increased temporarily when blood pressure dropped.

# **BILATERAL ADRENALECTOMY WITH IV HYDROCORTISONE (FREE ALCOHOL) PREPARATION**

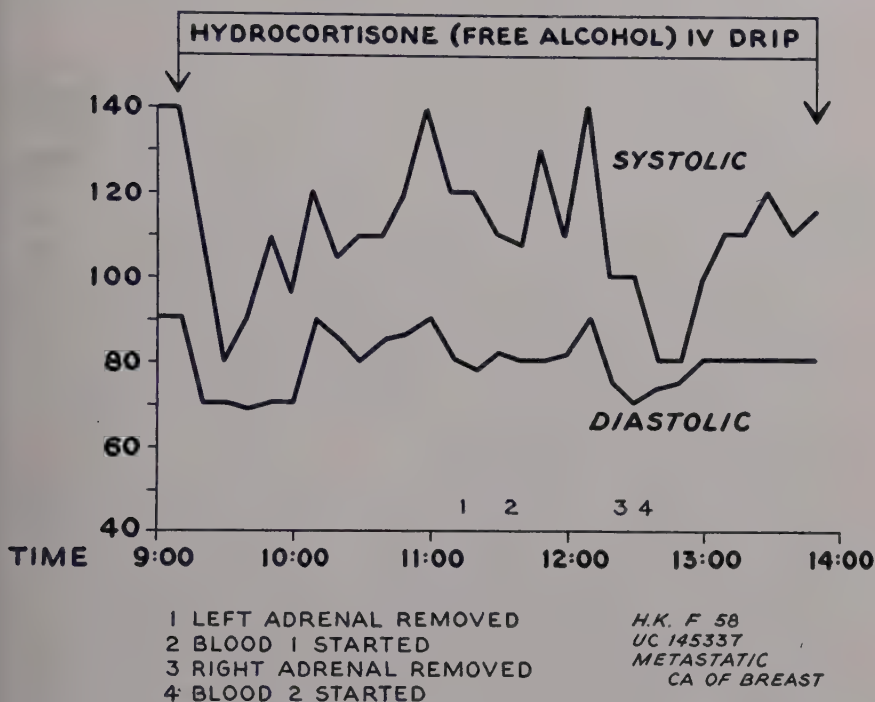


FIGURE 6. From Rukes, Orr, and Forsham.<sup>18</sup>

Transfusions in excess of blood loss or vasopressor drugs were not necessary. The postoperative course was smooth. In the afternoon, after the total bilateral adrenalectomy, hydrocortisone 25 mg. intramuscularly every six hours was started, and continued for the first two postoperative days. The management subsequent to this was standard.

(2) *Intravenous hydrocortisone in acute adrenal insufficiency following major surgery.* The crisis of acute adrenal insufficiency precipitated by stress or surgery in patients with Addison's disease, panhypopituitarism, functional adrenal insufficiency resulting from antecedent corticosteroid therapy (iatrogenic adrenal insufficiency), represents a logical indication for the use of intravenous hydrocortisone.

Continued administration of cortisone or hydrocortisone in doses exceeding 25 mg. daily will induce functional adrenal insufficiency, not only for some time after corticosteroid therapy has been discontinued, but also during maintenance therapy. The dose adequate to control the disease being treated may prove inadequate to meet the extra demands of acute, severe stress. With more widespread use of corticosteroids in the treatment of chronic diseases

the incidence of crises resulting from iatrogenic adrenal insufficiency will undoubtedly increase, unless it is anticipated and appropriate therapy instituted.

The following cases demonstrate the value of intravenous hydrocortisone in the treatment of acute adrenal insufficiency resulting from such adrenal suppression:

*Case 1, K. McE., U.C.H. No. 222184:* A 52-year-old white male with proved ulcerative colitis did not respond to conservative therapy. He was treated with cortisone acetate (25 mg. orally every six hours) and then hydrocortisone (40 mg. orally every six hours) with subjective but not objective improvement. After approximately one month of steroid therapy, the hydrocortisone dose was decreased in a stepwise manner and, simultaneously, a course of corticotropin (ACTH) in decreasing dosage was given. Six days after discontinuation of the ACTH, a colectomy was performed. During surgery, shock developed, which was not responsive either to blood transfusions or to the intravenous use of a vasopressor, methoxamine hydrochloride (Vasoxyl). Hydrocortisone (100 mg. in 1000 cc. 5 per cent dextrose in water) given as a slow intravenous drip led to a prompt return of blood pressure to normal limits. Normotension was maintained throughout the operation and the immediate postoperative period (TABLE 2).

*Case 2, A. L., U.C.H. No. 231601:* This 21-year old white male had been on medical therapy for chronic ulcerative colitis with a steady decline in weight. In preparing him for ileostomy, he was taken off ACTH (FIGURE 7) and placed on 120 mg. of oral hydrocortisone six days prior to surgery. On the day of surgery, he was given an infusion of 100 mg. of hydrocortisone but no further corticoid medication was administered on the first postoperative day. In an attempt to avoid poor wound healing, hydrocortisone was rapidly reduced from 80 to 0 mg. per day from the second postoperative day to the fifth. On the sixth postoperative day, shown on top of FIGURE 7, the patient went into shock during the afternoon and appeared acutely ill, demonstrating extreme generalized weakness. As it became apparent that the rapid reduction in hydrocortisone dosage, quite adequate in patients who did not have previous ACTH or corticoid therapy, had led to acute adrenal insufficiency in this patient, because of adrenal suppression prior to surgery, intravenous hydrocortisone was started. The patient improved generally within one half hour

TABLE 2  
BLOOD PRESSURE CHANGES IN PATIENT K. MCE. DURING SURGERY

Time after induction of anesthesia	Treatment	Blood pressure	
		Systolic	Diastolic
0	1 Unit blood started	80	60
2 hr.	2nd Unit blood started	90	50
3 hr. 25 min.	Vasoxyl <sup>R</sup> 10 mgm. I.V.	80	50
3 hr. 30 min.	Continued	70	45
3 hr. 35 min.	100 mgm. hydrocortisone started I.V.	60	45
3 hr. 40 min.	Continued	100	70
4 hr.	Continued	130	80



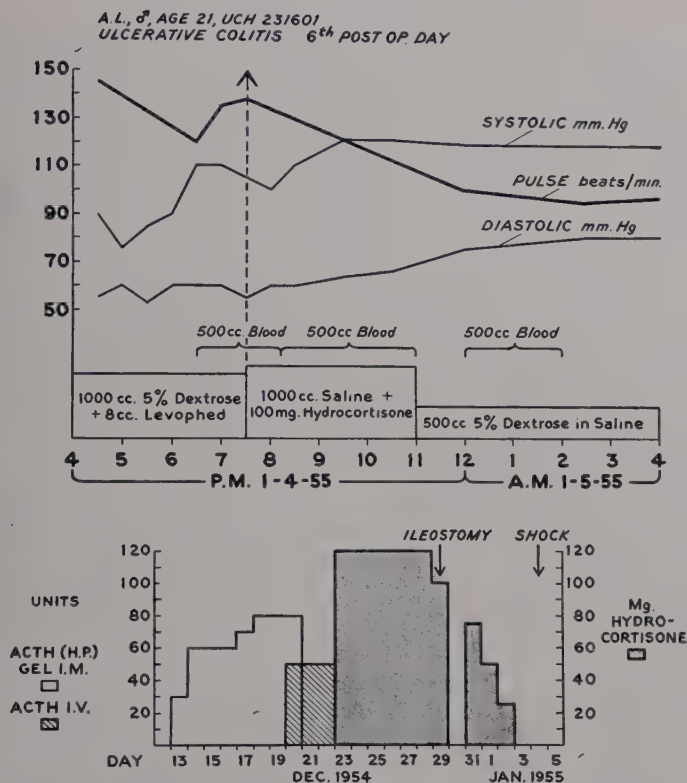


FIGURE 7. The use of hydrocortisone in combatting shock in a patient with iatrogenic adrenal insufficiency. The lower half of the figure shows the preceding therapy responsible for adrenal cortical unresponsiveness.

and the changes in vital indices approached normal within three hours, as shown in FIGURE 7.

*Shock unresponsive to standard therapy.* Several cases of shock occurring during surgery or postoperatively have been encountered which were not benefited by the usual therapy of blood replacement and vasopressor drugs, but did react dramatically to the administration of intravenous hydrocortisone. Adrenocortical extract has been shown to potentiate the action of vasoconstrictors on blood vessels.<sup>12</sup> The elevation of the tetraethylammonium floor by ACTH or cortisone is indirect evidence that the corticosteroids have a direct effect on blood vessels.<sup>13</sup> These basic considerations justify the trial of intravenous hydrocortisone, even where true adrenal insufficiency cannot be proved, in selected cases of shock which do not respond to transfusions and vasopressors.

The effectiveness of intravenous hydrocortisone in stubborn shock states is illustrated in the following cases:

*Case 1, C. F., U.C.H. No. 195839.* This 60-year-old white male had a total gastrectomy with esophagojejunostomy for repair of a large hiatus hernia and

an extensive carcinoma of the esophagus. Following this, nutrition had been a serious problem. On Feb. 12, 1954, a revision of the esophagojejunostomy was accomplished and a jejunal pouch formed. During this extensive procedure, multiple blood transfusions and both intravenous and intramuscular vasopressor agents were necessary to maintain blood pressure. After eight hours, and in spite of the administration of six units of blood, these measures ceased to be effective and an infusion of hydrocortisone was started. For the remainder of the operation and the immediate postoperative period the blood pressure was successfully maintained (FIGURE 8).

*Case 2, E. S., U.C.H. No. 218667*, a 30-year-old white female with rheumatic mitral valve disease underwent cardiac surgery Oct. 31, 1953. Marked mitral insufficiency was observed and valvuloplasty was not considered advisable. On the second postoperative day, the blood pressure ceased to respond to vasopressors and remained at 90/70 despite the continuous intravenous administration of norepinephrine. When an infusion of 100 mg. of hydrocortisone in 5 per cent dextrose was given, within two hours the blood pressure rose to

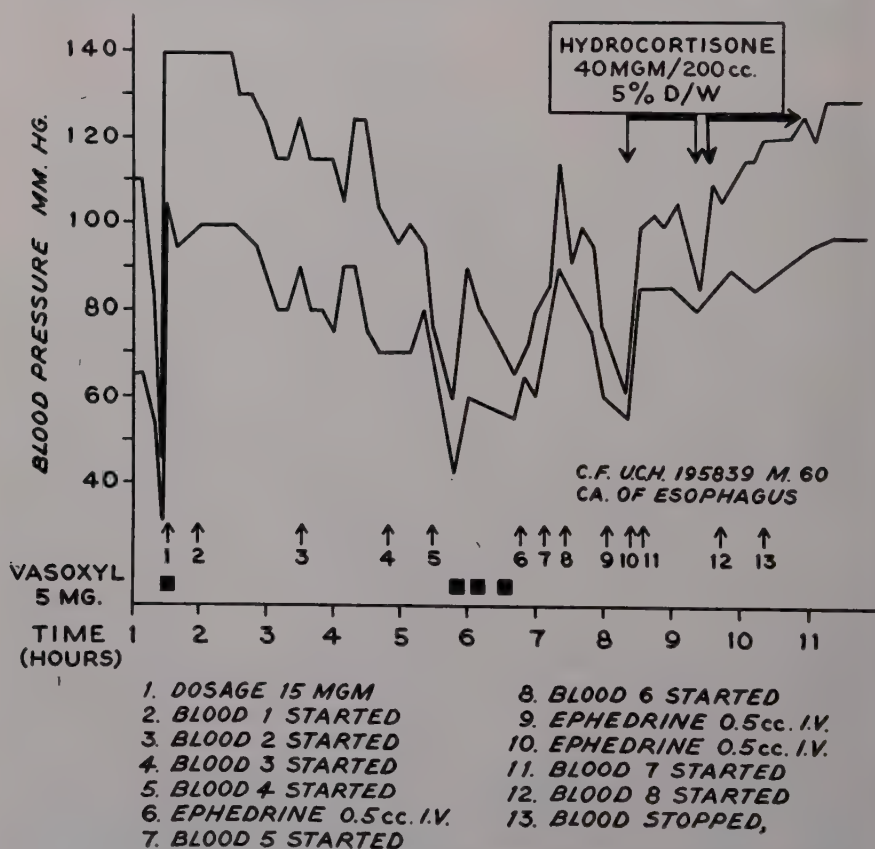


FIGURE 8. The use of intravenous hydrocortisone in combating postoperative shock unresponsive to conventional therapy. From Rukes, Orr, and Forsham.<sup>18</sup>

124/90 without further use of vasoconstrictors (FIGURE 9). For the next four days, hydrocortisone was given intramuscularly in decreasing doses with satisfactory maintenance of blood pressure.

The striking rise of blood pressure in this patient, who was started on intravenous hydrocortisone while intravenous norepinephrine was continued, may be due to the synergistic action between hydrocortisone and vasoconstrictor agents.<sup>12</sup>

*Other therapeutic uses.* Indications for the use of intravenous hydrocortisone include fulminating allergic and toxic reactions, such as anaphylactic shock; severe penicillin reactions;<sup>14</sup> and "thyroid storm."

*Precautions to observe.* Two possible dangers must be considered when using this form of the hormone for short-term, intensive therapy. Since hydrocortisone and cortisone increase the excitability of the central nervous system and lower the electroshock threshold in the rat,<sup>15</sup> an epileptic seizure may be precipitated in susceptible patients. If convulsions occur, intravenous barbiturates should be used. Another danger is the possibility of excessive sodium retention produced by a high sodium intake during the administration of intravenous hydrocortisone. Thorn *et al.*<sup>16</sup> have shown that considerable sodium retention occurs in a normal subject during a continuous intravenous infusion of 12 mg. hydrocortisone per hour in saline. The relatively short duration of the hydrocortisone infusion makes sodium retention and edema no

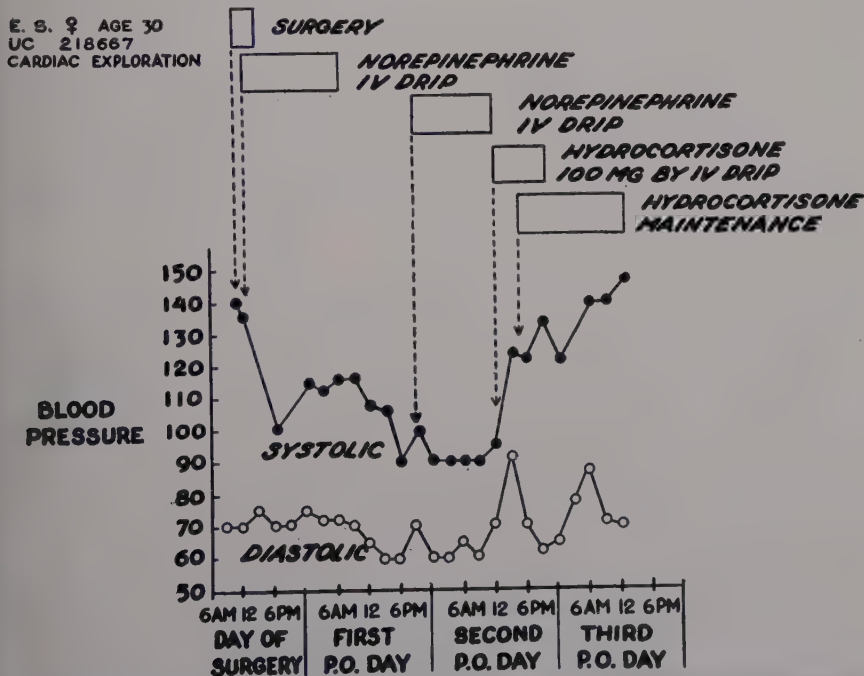


FIGURE 9. The use of intravenous hydrocortisone in the treatment of unresponsive postoperative shock, not related to blood loss in a patient following cardiac surgery. From Rukes, Orr, and Forsham.<sup>18</sup>

serious problem, except in cases where incipient heart failure exists. The use of 5 per cent dextrose in water rather than saline as a diluent for the concentrated hydrocortisone solution will minimize these dangers. Supplementary potassium should be given to patients already on prolonged steroid therapy, unless renal impairment contraindicates this.

*Experience with the use of intravenous hydrocortisone.* To date, more than 50 surgical cases have been given from 100 to 500 mg. of hydrocortisone at the University of California Hospital. There has been no case in which there was wound dehiscence attributable to this medication. When sepsis was present, it was controlled by adequate antibiotics. In nearly all of the cases, the use of the material has been limited to a five-day period after which the drug was either discontinued or lower dosages of cortisone were used.<sup>17</sup> Because of the life-saving properties this medication frequently exhibits, it should be used freely whenever the question of adrenal cortical insufficiency exists.<sup>18</sup> One should not wait for laboratory confirmation, which is unreliable in the case of the eosinophil count and should avoid too much delay in waiting for a determination of blood or urinary 17-hydroxycorticoids.

### Summary

Because of the protean metabolic effects and rapidity of action of hydrocortisone, it is the therapeutic agent of choice in the management of acute adrenal insufficiency. The use of intravenous hydrocortisone is indicated when the rapid attainment of high blood levels appears desirable. Intravenous hydrocortisone has proved effective and safe in the treatment of acute adrenal insufficiency, certain cases of shock unresponsive to standard therapy and severe allergic and toxic states associated with major surgery. Reasons for its relative safety when used over short periods of time with suitable precautions have been presented. The use of aqueous whole-adrenal extract is probably no longer justified with the availability of this soluble and potent hydrocortisone preparation.

### References

1. HECHTER, O. & G. PINCUS. 1954. Genesis of adrenocortical secretion. *Physiol. Revs.* **34**: 459.
2. ROMANOFF, E. B., P. HUDSON & G. PINCUS. 1953. Isolation of hydrocortisone and corticosterone from human adrenal vein blood. *J. Clin. Endocrinol. & Metabolism.* **13**: 1546.
3. AXELRAD, B. J., B. B. JOHNSON & J. A. LUETSCHER. 1954. Factors regulating the output of sodium-retaining corticoid of human urine. *J. Clin. Endocrinol. & Metabolism.* **14**: 783. (Abstract).
4. DOUGHERTY, T. F. 1953. Some observations on mechanisms of corticosteroid action on inflammation and immunologic processes. *Ann. N. Y. Acad. Sci.* **56**: 748.
5. BOLAND, W. E. 1952. Antirheumatic effects of hydrocortisone (free alcohol) hydrocortisone acetate and cortisone (free alcohol) as compared with cortisone acetate. *Brit. Med. J.* **1**: 559-564.
6. AMELUNG, D., H. J. HUBENER, L. ROKA & G. MAYERHEIM. 1953. Conversion of cortisone to compound F. *J. Clin. Endocrinol. & Metabolism.* **13**: 1125.
7. MACEK, T. J., W. H. BAADÉ, A. BORNH & F. A. BACHER. 1953. Observations on the solubility of some cortical hormones. *Science.* **116**: 399.
8. RICHARDS, J. B. & M. L. SWEAT. 1953. Peripheral blood concentration of steroids in man after oral administration of 17-hydroxycorticoids. *Proc. Soc. Exptl. Biol. Med.* **84**: 125-127.



9. NELSON, D. H., A. A. SANDBERG, J. G. PALMER & F. H. TYLER. 1953. Blood levels of 17-hydroxycorticosteroids following the administration of adrenal steroids and their relation to circulating leukocytes. *J. Clin. Invest.* **31**: 843.
10. COCHRANE, G. C., J. P. JOHN, N. FOREMAN & L. W. KINSELL. 1953. Evaluation of adrenal steroids administered intravenously, intramuscularly, and orally. *J. Clin. Endocrinol. & Metabolism.* **13**: 993.
11. HUME, D. M. 1953. The neuro-endocrine response to injury: present status of the problem. *Ann. Surg.* **138**: 548-557.
12. FRITZ, I. & R. LEVINE. 1951. Action of adrenal cortical steroids and nor-epinephrine on vascular responses to stress in adrenalectomized rats. *Am. J. Physiol.* **165**: 456.
13. BRUST, A. A., W. RANSOHOFF & M. F. REISER. 1951. Blood pressure responses to ACTH and cortisone in normotensive and hypotensive subjects in the resting state and during autonomic blockade with tetraethylammonium chloride. *J. Clin. Invest.* **30**: 630. (Abstract).
14. BOGER, W. P., W. B. SHERMAN, I. W. SCHILLER, S. SIEGEL & B. ROSE. 1953. Allergic reactions to penicillin: a panel discussion. *J. Allergy.* **24**: 383-404.
15. WOODBURY, D. N., C. P. CHENG, G. SAYER & L. S. GOODMAN. 1950. Antagonism of adrenocorticotrophic hormone and adrenalcortical extract to desoxycorticosterone, electrolytes, and electroshock threshold. *Am. J. Physiol.* **160**: 217.
16. THORN, G. W. *et al.* 1953. Pharmacologic aspects of adrenocortical steroids and ACTH in man. *New Engl. J. Med.* **248**: 232, 284, 389, 414, 588, and 632.
17. GALANTE, M., M. RUKES, P. H. FORSHAM & H. G. BELL. 1954. The use of corticotropin, cortisone, and hydrocortisone in general surgery. *Surg. Clin. North Am.* **34**: 1201.
18. RUKES, J. M., R. H. ORR & P. H. FORSHAM. 1954. Clinical uses of intravenous hydrocortisone. *Metabolism.* **3**: 481.

# THE USE OF INTRAVENOUS HYDROCORTISONE IN ASTHMA\*

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## *Introduction*

Intravenous hydrocortisone has proved of value in, at least, the three following phases of asthmatic therapy: "status asthmaticus" after the usual forms of therapy have failed; initial therapy in patients invalidated by severe, intractable asthma of undetermined etiology in whom maintenance steroid medication is contemplated; and the carrying through of major surgical procedures in patients whose symptoms of asthma have been suppressed for long periods by daily administration of compounds E or F. The following case reports, from records of the Massachusetts General Hospital (M.G.H.), illustrate each of these phases.

## *Case Reports*

*Case of H. S., white, male, 41 years—M.G.H. No. 858635.* He was well until February 1948 when, at age 35, he developed a chest cold. With this respiratory infection came his first asthma. Soon the cold cleared, but wheezing persisted until the spring of 1948 when it, too, disappeared. All was well until February 1949, when asthma reappeared following another cold. This time, the asthma became more severe. Soon thereafter, he began to frequent hospitals. For the following four years, he had severe daily asthma aggravated by respiratory infections. Pneumonia in June 1949 did not relieve him from asthma. In June 1953, corticotropin was given for the first time with only partial relief. During several upper respiratory tract infections, a combination of penicillin, corticotropin, and Demerol seemed temporarily effective. The patient stated that cortisone, both oral and intramuscular, had been tried without success in the past, but its dosage was unfamiliar to him. In January 1954, he went to Florida, where he soon began to board in hospitals. Finally, in May 1954, he returned home, only to enter a hospital, where he almost died of asthma. Following this admission, he attempted to control his symptoms with 0.5 ml. ACTH gel intramuscularly daily and 100 mg. of Demerol daily. The record was not marked with success.

On Aug. 25, 1954, at 4:30 P.M. he was admitted to the Massachusetts General Hospital in "status asthmaticus." His condition was considered serious. He was placed on an intravenous drip of ACTH, 40 units, and epinephrine 1-1000, 1 ml. in 1500 ml. of 5 per cent glucose in water. Later 200 mg. of Demerol were added. He did not do well.

On Aug. 26, 1954, his intravenous drip was changed to 300 mg. hydrocortisone in 1500 ml. of 5 per cent glucose in water. This infusion ran in over an eight-hour period. At the end of this treatment, 200 mg. hydrocortisone in 1000 ml.

\* Supported by a grant from the Upjohn Company, Kalamazoo, Mich. Intravenous hydrocortisone (Cortef) supplied by the Upjohn Company.

† The authors are indebted for technical assistance to Priscilla Gordon and Mary Gilchrist.

of 5 per cent glucose in water were given in like manner. He was much improved on the morning of Aug. 27, 1954. He was then given 200 mg. hydrocortisone in 1500 ml. of 5 per cent glucose in water every 12 hours by slow intravenous drip. On this same day, he began to cough up enormous amounts of thick, tenacious sputum in quantities sufficient to fill several sputum cups in one day. Actual bronchial casts were found in this material. On Aug. 28, 1954, he was changed to 60 mg. of oral hydrocortisone every six hours. He improved steadily and left the hospital on Sept. 4, 1954, completely free of asthma on 30 mg. oral hydrocortisone every eight hours.

Complete laboratory studies throughout his hospital stay showed only one point of interest. During his intravenous course of hydrocortisone and, for a few days afterward, his 24-hour urines showed a large calcium excretion (over 200 mg. every 24 hours).

*Case of G. Mac., white, male, 56 years—M.G.H. No. 857222.* This patient's asthma first appeared in 1936, at age 38. From then on, he had persistent asthma wherever he went and during all seasons. In the fall of 1953, while hunting in Maine, emergency hospitalization was necessary. At that time, initial cortisone was most effective, but after two months of inadequate maintenance therapy, asthma became severe again, so therapy was discontinued. Between January 1954 and his admission to the Massachusetts General Hospital on April 20, 1954, he was able to work only two weeks.

On admission, he was found to have severe asthma. Attempts to find responsible antigens were unsuccessful. Physical examination showed him to have moderate emphysema. Aminophylline, epinephrine, and other routine measures failed to give adequate relief. On May 2, 1954, he was started on a slow intravenous drip (200 mg. of hydrocortisone in 1000 ml. 5 per cent glucose in water). Within a few hours, he showed marked improvement. When the intravenous drip was finished, he was started on 50 mg. of oral hydrocortisone every six hours. He became completely free of asthmatic symptoms after a total dose of one gram of hydrocortisone.

*Case of F. S., white, female, 46 years—M.G.H. No. 335119.* As an infant, she had atopic eczema. She then cleared, and was well until age 37, when she again developed eczema of the popliteal and antecubital spaces, intermittent in character. In October 1951, at age 43, asthma appeared and was intractable from its onset. Attempts to discover responsible antigens were unavailing. Intensive therapy, including hospital care, did not control her symptoms. Her course was toward chronic invalidism. On Jan. 29, 1952, oral cortisone was started. Her progress was so satisfactory that she was maintained essentially symptom-free on daily doses of cortisone for the next 15 months on a dosage schedule averaging 75 mg. every 24 hours.

In May 1953, she developed symptoms of cholelithiasis. Roentgenograms confirmed the diagnosis. A medical regime was not too successful. Therefore, in March 1954, she was readmitted to the hospital for cholecystectomy. Hydrocortisone 100 mg. in 1000 ml. 5 per cent glucose and water was started as a slow intravenous drip (40 drops/minute) at 6:30 A.M. on March 12, the morning of operation. At 8 A.M., she was taken to the operating room.

Surgery was uneventful. At 6 P.M. that evening 100 mg. hydrocortisone in 1000 ml. 5 per cent glucose was again started as a slow intravenous drip. This infusion was repeated at 6 A.M. the following morning, March 13. She did well. On March 14, she returned to 25 mg. oral cortisone every 8 hours. Recovery was normal. Neither asthma nor shock became a problem. No blood transfusion was given.

### *Discussion*

Whenever a new therapeutic agent is presented, its true worth depends on at least three factors. First, is it effective? Second, is it safe? Third, does it offer advantages over present medications? Such questions can not be answered fully in the short period in which hydrocortisone has been available in intravenous form, but work to date points to its success in fulfilling the above qualifications.

During the past year, intravenous hydrocortisone has been used in over 60 patients on this service. It has proved a most effective agent. The cited cases represent the general rule, not the exceptions.

To date, intravenous hydrocortisone has proved to be a reasonably safe agent if handled with care and used for relatively short periods of time. There has been however, one serious complication, possibly due to hydrocortisone. An elderly white man was admitted in carbon dioxide narcosis. His history was one of asthma of many years duration. There was no history suggestive of peptic ulcer. This patient was found to have marked pulmonary fibrosis with only a mild bronchospastic element. Studies indicated, however, that his main problem was based upon a lack of functional pulmonary tissue. On administration of oxygen, he ceased breathing, as his oxygen lack had been responsible for his respiratory efforts. Positive pressure breathing was not successful. Subsequent transfer to a respirator was followed by improvement. After five days, his course was not encouraging, as he had been in and out of CO<sub>2</sub> narcosis several times. In spite of 24-hour nursing and medical supervision, it became obvious that the end was near. It was decided that compound F might relieve the existing bronchospasm and that it might also have a beneficial effect on the small pulmonary blood vessels. He was placed, therefore, on a slow intravenous drip of 200 mg. hydrocortisone in 1000 ml. of 5 per cent glucose and water. He did improve. Unfortunately, however, he developed massive bleeding from the gastrointestinal tract on the following day, whereupon hydrocortisone was promptly discontinued. Subsequently he died, and autopsy showed a large gastric ulcer. A question must arise here as to whether the repeated episodes of CO<sub>2</sub> narcosis or the hydrocortisone were responsible for his bleeding ulcer. While this question can not be conclusively answered, it seems fair to remember the possible responsibility of hydrocortisone in this situation.

Prior to the advent of intravenous hydrocortisone, intravenous corticotropin was probably the agent of choice in the three phases of asthma just discussed. Compound F, as an intravenous agent, seems to have at least two theoretical advantages over intravenous ACTH. At the present time, commercial prepa-



rations of corticotropin contain protein, and therefore are more likely to cause allergic reactions than is hydrocortisone, a synthetic compound. To date, the literature supports this observation with numerous authors<sup>1, 2, 3, 4, 5</sup> reporting anaphylactic type of reactions with ACTH in contrast to rare reactions<sup>6</sup> to the steroids. Grolnick<sup>7</sup> failed to sensitize the skin of patients with cortisone. Evidence so far suggests that hydrocortisone may well be nearer the final effective agent than corticotropin or cortisone. In this clinic, intravenous hydrocortisone has proved more effective than intravenous ACTH. Intravenous hydrocortisone is also considered safer to use during operative procedures on patients who have been maintained for long periods on steroid therapy or in those who have had ACTH, cortisone, or hydrocortisone medication in the past but have discontinued their use within the calendar year. It is felt that atrophy of both the adrenals and the pituitary in such cases may delay the stimulating effect of ACTH at a time when rapid supportive action is necessary.

### *Summary and Conclusions*

Intravenous hydrocortisone has proved effective in three phases of asthmatic therapy: "status asthmaticus"; initial therapy in patients invalidated by severe intractable asthma of undetermined etiology in whom maintenance steroid therapy is contemplated; and the carrying through of major surgical procedures in patients whose symptoms have been suppressed for long periods by daily administration of compounds E or F.

### *References*

1. FEINBERG, S. M., A. R. FEINBERG & E. BIGG. 1951. Allergy to pituitary corticotrophic hormone. *J. Am. Med. Assoc.* **147**: 40.
2. WEST, H. F. 1951. New methods with ACTH and cortisone. *Lancet.* **2**: 226.
3. WILSON, L. 1951. Protein shock from intravenous ACTH. *Lancet.* **2**: 478.
4. WEST, H. F. & G. R. NEWNS. 1952. Allergy to bovine ACTH. *Lancet.* **1**: 1308.
5. SHULMAN, L. E., E. H. SCHOENRICH & A. MC. HARVEY. 1953. Allergic reactions to therapeutic agents: treatment with adrenocorticotrophic hormone (ACTH) or cortisone. *Bull. Johns Hopkins Hosp.* **92**: 196.
6. BERNSTEIN, D. 1951. Nasal and cutaneous allergy to cortisone. *N. Y. State J. Med.* **51**: 1849.

# THE ROLE OF THE ADRENAL CORTEX IN GLUCOSE AND PYRUVIC ACID METABOLISM IN MAN INCLUDING THE USE OF INTRAVENOUS HYDROCORTISONE IN ACUTE HYPOGLYCEMIA\*

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## *Introduction*

With Thomas Addison's observations, in 1855, of the disease which now bears his name, great interest was generated in determining its cause and treatment. The synthesis of the first adrenal steroid, desoxycorticosterone, by Reichstein in 1937, with its strong electrolyte-regulating effects, prolonged the lives of Addisonians and led to a search for other compounds with which to correct the remaining metabolic disturbances characteristic of the adrenal deficient state. Newly isolated steroids from natural sources were shown to exert profound carbohydrate effects such as hyperglycemia, glycosuria, and hepatic glycogenesis. Ingle<sup>1</sup> observed glycosuria in normal, force-fed rats given 17-hydroxycorticosterone, 11-dehydro-17-hydroxycorticosterone,<sup>2</sup> corticosterone,<sup>3</sup> and adrenocorticotropin hormone.<sup>4</sup> Forsham<sup>5</sup> and Perera,<sup>6</sup> using 11-dehydrocorticosterone, prevented hypoglycemia in fasted Addison disease patients without producing any significant increase in the fasting blood sugar or glucose tolerance. Eventually, a hierarchy of steroids was established which graded the steroids according to their electrolyte-effects on the one hand, and their carbohydrate effects on the other. Interestingly, the steroids possessed opposite degrees of potency when compared by these two standards of biological activity.

The effect of certain of the adrenal steroids upon the glycogen content of the liver is very marked. According to Pabst, Sheppard, and Kuizenga,<sup>7</sup> 17-hydroxycorticosterone is the most potent in causing deposition of liver glycogen, followed by 11-dehydro-17-hydroxycorticosterone, corticosterone, and 11-dehydrocorticosterone. The compound 11-desoxycorticosterone is only weakly glycogenic, although it is the most potent in its effect upon electrolyte balance.

With this knowledge, there developed an interest in determining other sites of action of the carbohydrate-active steroids. The ameliorating effect of adrenalectomy in diabetes of animals was shown by Long and Lukens.<sup>8</sup> The Coris suggested an inhibitory action of these steroids on the glucokinase reaction of carbohydrate synthesis. Cortical hormones participate in the synthesis of carbohydrate from proteins (gluconeogenesis), in which certain amino acids are deaminated and then combined into six-carbon chains to form glucose. A second function is to influence the reaction  $\text{glucose} \rightleftharpoons \text{glycogen}$  towards the right, increasing the storage of glycogen in the liver at the expense of the blood sugar. In this reaction, many other hormones have an influence: insulin likewise promotes glycogen storage, while thyroxine and epinephrine mobilize glucose for use in the tissues. Anterior pituitary growth hormone antagonizes

\* These studies were made possible, in part, through the help and cooperation of the Upjohn Company, Kalamazoo, Mich.; Armour Laboratories, New York, N. Y.; and Ciba Pharmaceutical Products, Inc., Summit, N. J.

gluconeogenesis but promotes glycogen storage in muscle. A third action is the "anti-insulin" or diabetogenic action. Hyperadrenal patients are likely to become hyperglycemic, as are animals overtreated with cortical hormones. Gluconeogenesis does not alone explain the hyperglycemia, for nitrogen elimination, as in rats, is not sufficient for so much gluconeogenesis. Either another source, probably fat, is being utilized to produce carbohydrate, or the tissues are failing to withdraw their normal share of glucose from the blood to oxidize it, in which event cortical hormone is properly regarded as "diabetogenic" or "anti-insulin." Increasing evidence has accumulated pointing to an anti-insulin effect of adrenal steroids.

The purpose of the studies to be reported has been two-fold: first, to determine whether intermediates of carbohydrate metabolism may be altered in man by adrenal hormones and, second, to obtain information concerning the differences between "steroid" diabetes and "pancreatic" diabetes. During the course of these investigations, an opportunity to study the effect of intravenous hydrocortisone in hypoglycemia provided valuable additional information bearing on the problems under study.

#### *Effect of Adrenal Steroids on Glucose Tolerance*

Long and Lukens<sup>8</sup> were the first to show conclusively that adrenalectomy decreases the severity of pancreatic diabetes. Glycosuria is diminished, and there is a marked increase in insulin sensitivity. Long<sup>9</sup> also observed that the glycosuria of the partially pancreatectomized rat could be intensified by cortisone, and Ingle<sup>2</sup> demonstrated the production of glycosuria in force-fed normal animals given cortisone. The abnormal glucose metabolism accompanying Cushing's syndrome is corrected following any one of the several therapeutic procedures which reduce pituitary-adrenal function in this disease. Hypoglycemia, presumably due to temporary hyperinsulinism, may follow withdrawal of corticotropin or cortisone after prolonged administration.

Corticotropin and cortisone or cortisonelike steroids antagonize insulin peripherally and increase glycogen deposition in the liver. This increases the demand for insulin, which is met adequately in most patients.<sup>10</sup> FIGURE 1 depicts a single patient who received nine weeks of continuous ACTH therapy. Glucose tolerance tests obtained during the course of therapy showed no significant changes. Pyruvic acid alterations occurred which will be discussed below. Other patients unable to meet this demand develop diabetes, probably due to the prolonged insulin requirement and subsequent islet exhaustion. Conn<sup>11</sup> administered ACTH to normal subjects who, after only five to ten days, developed fasting hyperglycemia, glycosuria, and diminished glucose tolerance. This diabetes was also characterized by a renal type of glycosuria, insensitivity to insulin, and complete reversibility upon discontinuing ACTH. Patients with pre-existing diabetes have intensification of glycosuria and an increased requirement for insulin with corticotropin or steroid therapy. Fajans and Conn<sup>12</sup> have devised a cortisone-glucose tolerance test as a means of detecting potential diabetes in the nondiabetic relatives of diabetics.

Thirty-one of thirty-three patients with Cushing's syndrome described by

# SERIAL CHANGES in BLOOD PYRUVATE - LACTATE RESPONSE to I.V. GLUCOSE during ACTH THERAPY

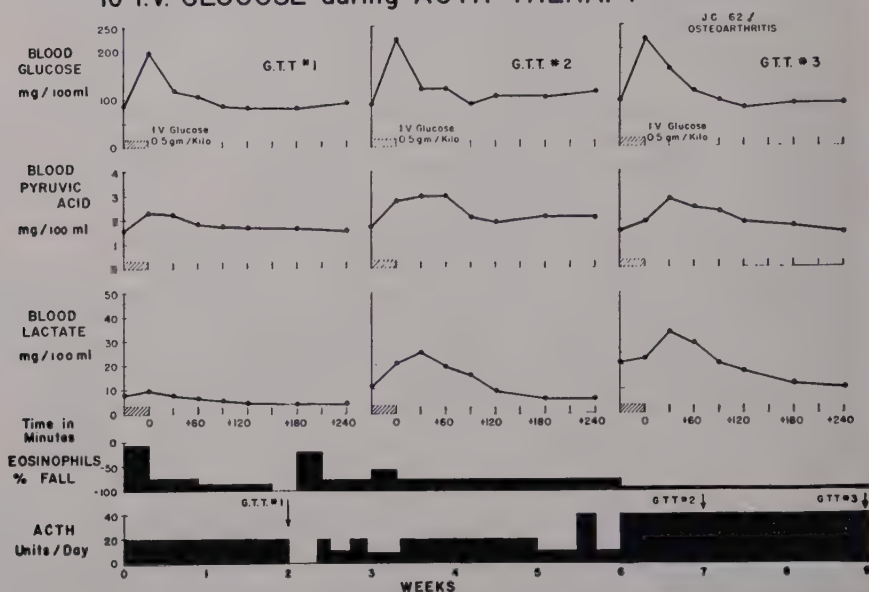


FIGURE 1. Repeated glucose tolerance tests (G.T.T.) in the same patient during prolonged corticotropin therapy did not change. The pyruvic acid levels increased.

Plotz *et al.*<sup>13</sup> exhibited diabetic glucose tolerance curves, but only nine had glycosuria, and frank diabetes was present in only five. In FIGURE 2, a diabetic glucose tolerance is shown in a patient with Cushing's syndrome. Some evidence of the severity of the islet deficiency is apparent from the persistence of the glucose defect three weeks after bilateral adrenalectomy. To the author's knowledge, although permanent diabetes is theoretically possible, it does not persist in Cushing syndrome after a remission in the disease is produced by any one of several means.

The carbohydrate activity of 11-oxysteroids is even more strikingly demonstrated in subjects who have coexisting Addison's disease and diabetes mellitus. Administration of cortisone causes a pronounced increase in the excretion of glucose, nitrogen, and ketone bodies, and an increase in the requirement for insulin.

## Adrenal Steroids and Pyruvate Metabolism

Pyruvic acid and lactic acid accumulate in the blood and tissues when carbohydrate is being metabolized at an increased rate, in anoxia and following exercise. Friedemann<sup>14</sup> has suggested that the blood lactate-pyruvate relationship may reflect the relative oxidative conditions of the body. Bueding<sup>15</sup> observed in normal individuals a significant rise in lactic and pyruvic acids after glucose administration, reaching a maximum at the end of the first hour and returning to normal in two to three hours. In patients with diabetes mellitus,



# ADRENAL CORTICAL FUNCTION and the RESPONSE to INTRAVENOUS GLUCOSE TOLERANCE TEST

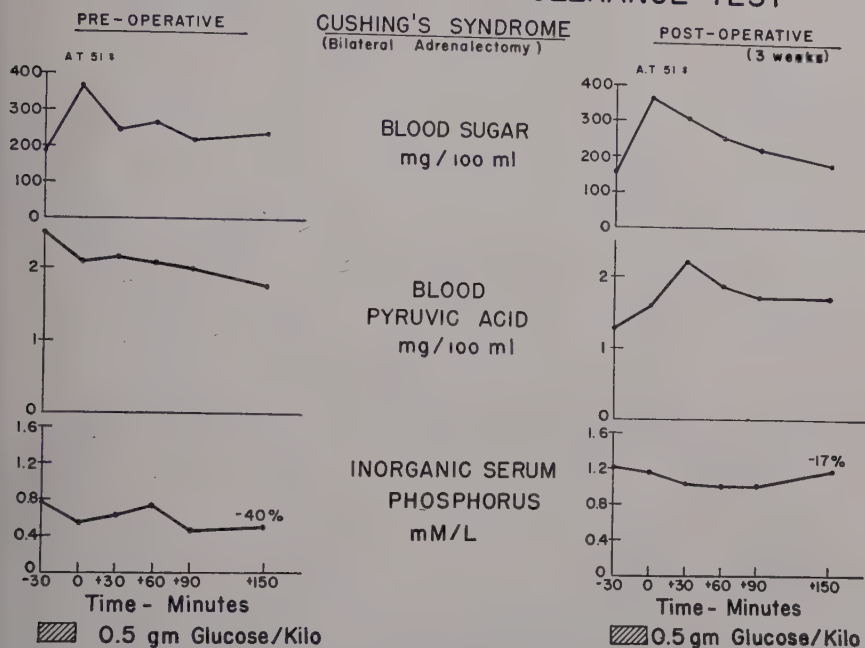


FIGURE 2. The diabetic type of glucose tolerance persisted after bilateral adrenalectomy. Markedly abnormal pyruvic acid levels were observed before and after surgery.

this rise was either absent or delayed, but the concentration of these substances could be increased by administering insulin.

Very few observations have been made on the relationship of adrenal function to lactic acid and pyruvic acid metabolism. Pyruvic acid rises in men suffering from chronic malnutrition and exposure to many other stressors. Lewis *et al.*<sup>16</sup> found that cortisone but not desoxycorticosterone restored the ability of adrenalectomized phlorrhizinized rats to form glucose from lactic and pyruvic acids and from alanine. It has been reported in animals that the blood lactate and pyruvate is low in adrenal insufficiency. In untreated Addison's disease (FIGURE 3), we have observed normal fasting levels of pyruvate and lactate, although the lactate-pyruvate ratio was 10.9, which is above the upper limit of normal, 9.3. Following replacement therapy with sodium chloride and cortisone, there was no significant change in fasting levels of these metabolites. In panhypopituitarism (FIGURE 4), the fasting pyruvate was above normal, and lactate fell in the normal range. These observations and others we have made do not indicate any consistent alteration of pyruvate metabolism in adrenal cortical insufficiency in man and are, therefore, at variance with observations made in adrenal insufficient animals.

We have observed a relatively frequent abnormality in pyruvic acid in situa-

## Metabolic Response Patterns Following Intravenous Glucose in Addison's Disease

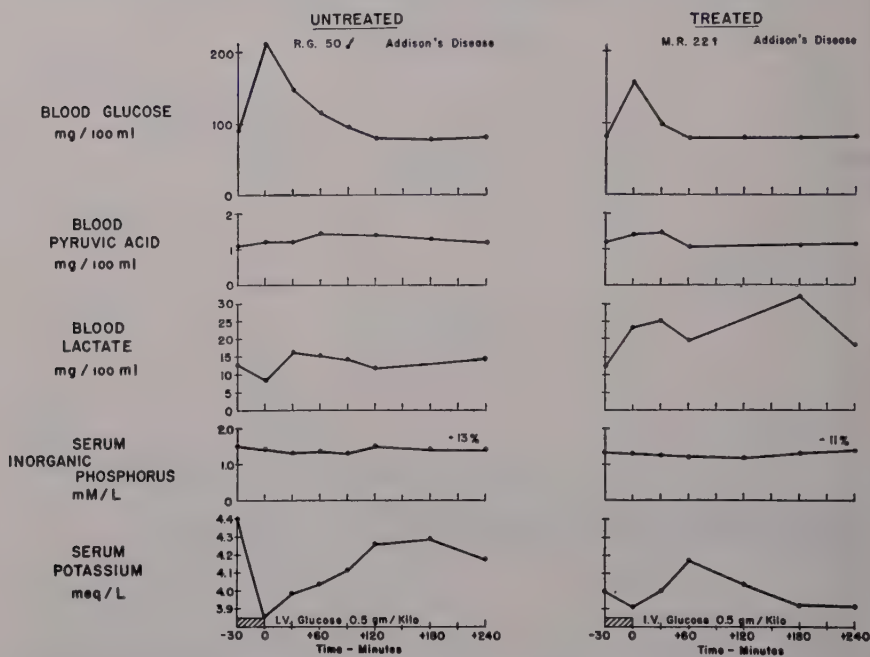


FIGURE 3. The response of two Addison's disease patients is compared. The untreated patient developed hypoglycemic symptoms after 180 minutes without showing a markedly lowered blood sugar. The fasting pyruvic acid was normal in both instances.

tions of adrenal cortical hyperfunction. An increase of blood pyruvic acid has been observed in: (1) Cushing's syndrome with diabetes; (2) in diabetes following prolonged corticotropin or 11-oxysteroid therapy; and, (3) in either (1) or (2) independent of any detectable alterations in glucose tolerance. In each instance, the elevated fasting blood pyruvate may represent an excess of insulin due to pancreatic stimulation by a persistently elevated blood sugar. FIGURE 2 shows a patient with Cushing's syndrome who had an abnormally elevated fasting pyruvic acid and diabetic glucose tolerance prior to operation. The lack of rise in pyruvic acid after glucose was characteristic of diabetes. Following bilateral adrenalectomy, the glucose tolerance was unchanged, but the fasting pyruvic acid was lower and a rise in pyruvic acid occurred following glucose. This is interpreted as an evidence of some restoration of peripheral insulin activity which is not apparent from the blood-sugar response alone.

Several patients have been studied during corticotropin or steroid therapy. Two different metabolic patterns of glucose and pyruvic acid have been observed. The majority of patients show no change in fasting blood pyruvate in spite of prolonged therapy in moderate doses and no abnormality in glucose tolerance. Others who receive large doses of therapy, particularly those developing Cushing's syndrome, show abnormally elevated fasting blood pyruvate

## Metabolic Response Patterns Following Intravenous Glucose in Pituitary Insufficiency

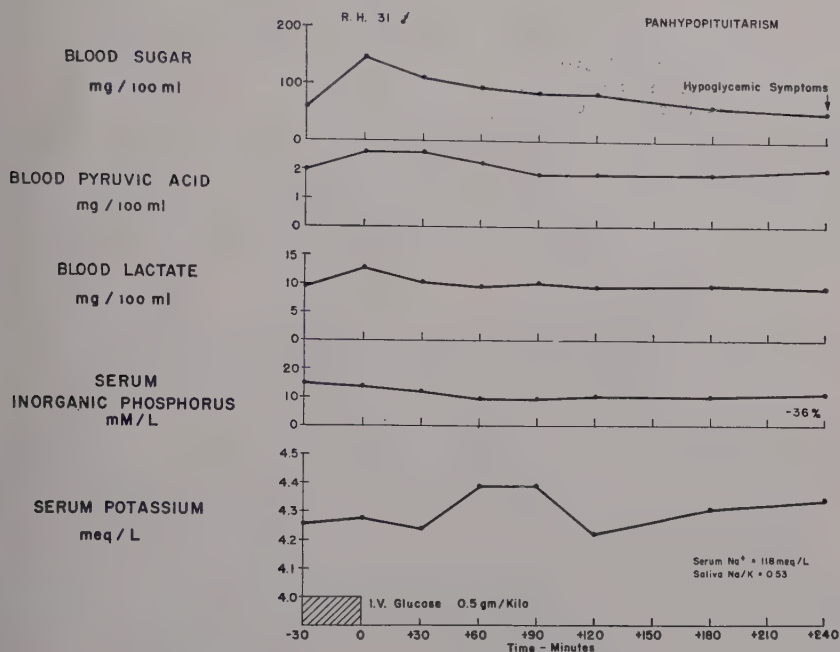


FIGURE 4. Hypoglycemic symptoms although delayed appeared without a marked reduction in blood sugar. The fasting pyruvic acid was elevated.

levels, often without any significant alteration in glucose tolerance (FIGURE 1). In both groups, however, it is interesting to note that, with or without changes in fasting pyruvate, there is a prolonged elevation of the pyruvic acid response to intravenous glucose, although glucose removal appears unimpaired. Other investigators have also observed alterations in pyruvic acid in either Cushing's syndrome<sup>17</sup> or following corticotropin or cortisone therapy.<sup>17-19</sup>

Although the measurements were not obtained under identical circumstances, an increase in pyruvic acid was observed uniformly. These are summarized in TABLE 1. Keyes and Kelley<sup>20</sup> observed, in dogs given adrenal cortical extract, greatly elevated blood pyruvic acid after an injection of glucose.

In 1950, Wilson, Frawley, and Forsham<sup>10</sup> expressed the opinion that there is a large functional reserve of islet tissue in the majority of patients who receive corticotropin or cortisone for therapeutic purposes, and that this reserve accounts for the infrequent occurrence of a significant impairment of carbohydrate tolerance. It was also suggested that a compensating hypofunctional state of the adrenals exists in diabetes.<sup>10</sup> Later, Field and Marble found reduced adrenal cortical reserve in diabetic patients.<sup>21</sup> Talbot *et al.*<sup>22</sup> found the urinary corticoid values in four controlled diabetic subjects in the same range

TABLE 1  
EFFECT OF ACTH AND CORTISONE ON PYRUVIC ACID (PA) METABOLISM

Author	Clinical state	Pyruvic acid sample	Pyruvic acid change and number of patients
Kerppola	Rheumatoid arthritis, bronchial asthma	Fasting after oral glucose	Increased (22/33)
Gitelson	Rheumatoid arthritis, ulcerative colitis	Fasting	Increased (17/19)
Lövgren	Rheumatoid arthritis	Fasting	Increased (10/10)
Hills Kerppola	Cushing's syndrome Cushing's syndrome	Fasting Fasting after oral glucose	Increased (4/11) Increased (5/5)
Frawley	Rheumatoid arthritis (ACTH-cortisone) Cushing's syndrome Addison's disease	Fasting and after i.v. glucose	Increased (3/3) Increased (1/1) Increased (2/3)

In a variety of disease states, the presence of increased adrenal steroids is accompanied by an elevated blood pyruvic acid.<sup>17-19, 86</sup>

as hypopituitary subjects and lower than in Addisonian patients. Forbes,<sup>23</sup> as well as Miller and Mason,<sup>24</sup> reported 17-ketosteroid values in diabetic patients somewhat lower than normal.

The observations on pyruvic acid noted above raise for consideration the possibility that an excess of 11-oxysteroids, either spontaneous or therapeutically induced, may increase pyruvic acid due to an accelerated conversion of glucose to pyruvate or, possibly, by interfering with its removal. Other factors to be considered in interpreting this effect of 11-oxysteroids on pyruvate metabolism are discussed below.

#### *Effect of Intravenous Steroids on Carbohydrate and Pyruvate Metabolism*

In studies designed to evaluate the effect of the adrenal cortex on metabolic processes, Ingle devised a technique of constant infusion of adrenal steroids into adrenalectomized animals throughout an experimental period.<sup>25</sup> This was believed to simulate as nearly as possible the manner in which the adrenal cortices secrete their hormones. As a result of numerous studies, Ingle concluded that the adrenal cortex did not initiate the metabolic changes accompanying stress or injury, but acted in a catalytic fashion in determining the direction, the degree, and rate of certain metabolic processes. He showed that adrenalectomized animals, given a uniform intake of adrenal cortex extract, had a normal metabolic response to injury. The metabolic response was not caused by the adrenal cortex, although the presence of cortical hormone was essential to support the overt manifestations of response. Ingle also suggested<sup>26</sup> that the comparison of the response of nonadrenalectomized animals with that of adrenalectomized animals on a fixed intake of cortical hormones would be useful in elucidating the relationship of adrenal cortex function to a biologic response.

In studies directed toward evaluating some of the metabolic adjustments occurring in man during stress, we adapted the Ingle technique to man with the exception that the majority of subjects studied to date have had intact adrenals. In our first studies<sup>27</sup> using a constant intravenous infusion of steroids,



solutions of free cortisone and free hydrocortisone were prepared according to the technique of Thorn with sterile absolute or 95 per cent alcohol and water.<sup>28</sup>

In later studies, intravenous hydrocortisone (Cortef\*) was employed. During the intravenous infusion an intravenous glucose tolerance test was performed and, in some studies, pyromen was added as a stressor. It should be stated that considerable controversy exists as to whether glucose *per se* is a stress agent capable of stimulating adrenal cortical activity. Recant<sup>29</sup> failed to produce a significant fall in circulating eosinophils in eight normal subjects after the intravenous injection of glucose. Skelton<sup>30</sup> found no adrenal cholesterol or ascorbic acid depletion in rats after glucose. Others, however, using lymphocyte depression and eosinopenia as indices, consider glucose as a stress agent.<sup>31</sup> Schneeberg,<sup>32</sup> in a comprehensive review of all available evidence, concludes that intravenous glucose, given for short periods of time, does not act as a stress agent and, therefore, does not produce a discharge of adrenal steroids. Although we take a similar position, it is by no means definitive because, until quantitative measurements of the circulating adrenal steroids and their metabolites are obtained following glucose, it is necessary to have some reservations as to whether glucose provokes an adrenocortical response or not.

As a preliminary to the studies with glucose and pyromen, it was necessary to establish the time required for an infusion of steroids to reach a relatively constant level of circulating adrenal steroids. Hydrocortisone was made up in 20 per cent alcohol added to 500 ml. of isotonic saline and infused at a rate of 12.5 mg. or 25 mg. per hour. Urine excretion measurements of 17-hydroxycorticoids, according to the method of Reddy, showed that there was a steady rise in steroid excretion which reached a relatively constant level after approximately two hours of infusion.<sup>33</sup> Reddy also showed that, at a dosage rate of 12 mg. per hour, there was a lag period of one hour, followed by a steady rise in output of corticoid until a plateau between 1.0 and 1.2 mg. per hour is reached. Infusions of hydrocortisone, at rates of 12.5 mg., 25 mg., and 50 mg. per hour, confirmed Reddy's findings that the excretory rate was relatively constant after two hours (FIGURE 5). It was assumed, therefore, that, by the third hour, the tissues had also reached a steady state, and that the circulating level of steroids was relatively constant. At this time, an intravenous glucose tolerance test was performed, using 0.5 gm. of glucose per kilogram of body weight infused over a 25- to 30-minute period.

In normal subjects, the infusion of hydrocortisone at a rate of 12 mg. per hour caused a significant fall in serum phosphorus, an increase in urine glucose, and progressive eosinopenia. There was no effect on glucose tolerance, however, when compared to a control tolerance test performed in the absence of hydrocortisone (FIGURE 6). Increasing the hydrocortisone to 25 mg. per hour produced similar effects without influencing the glucose tolerance (FIGURE 7). Definite, although not marked, changes in pyruvate levels were observed. The pyruvic acid response to glucose was not impaired by the intravenous steroids and, indeed, was actually prolonged when compared to the control.

In other experiments, pyromen was given 90 minutes after beginning a con-

\* Generously supplied through the courtesy of Doctors Neil O'Donovan and H. Hailman of the Upjohn Company, Kalamazoo, Mich.

# URINARY 17-HYDROXYCORTICOID EXCRETION DURING CONSTANT INTRAVENOUS HYDROCORTISONE (FREE)

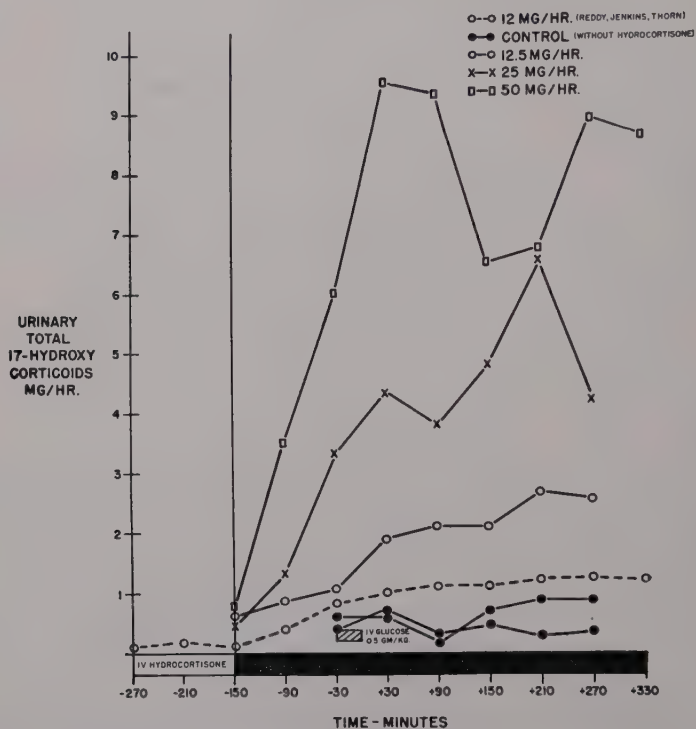


FIGURE 5. The level of urinary 17-hydroxycorticoids (Reddy<sup>33</sup>) was relatively constant after two hours at various dose levels of intravenous hydrocortisone. Intravenous glucose alone had no effect on the level of urinary steroids.

stant infusion of hydrocortisone. A dosage of 10 gamma was given intravenously. Thirty minutes later the glucose tolerance test was begun (FIGURE 8). The glucose tolerance, when compared to the control and during hydrocortisone alone, was slightly elevated, but the pyruvic acid response remained elevated for a considerably longer period. The effect of the pyromen was to accentuate the abnormality of the pyruvate response. Instead of a lack of pyruvate response typical of diabetes mellitus, the pyruvic acid rose and remained elevated, similar to the pyruvate response observed in patients receiving corticotropin or cortisone. This suggests that the altered glucose tolerance observed in patients treated with these agents is due to a stress imposed by the illness under treatment which is superimposed on a normal or increased level of tissue and circulating adrenal hormone. An altered glucose tolerance is not due solely to adrenal factors.

An opportunity to observe the effect of the stress of hypoglycemia on pyruvate response occurred in a patient with hyperinsulinism due to a pancreatic

## The Effect of a Constant Infusion of Hydrocortisone on the Response Pattern to I.V. Glucose

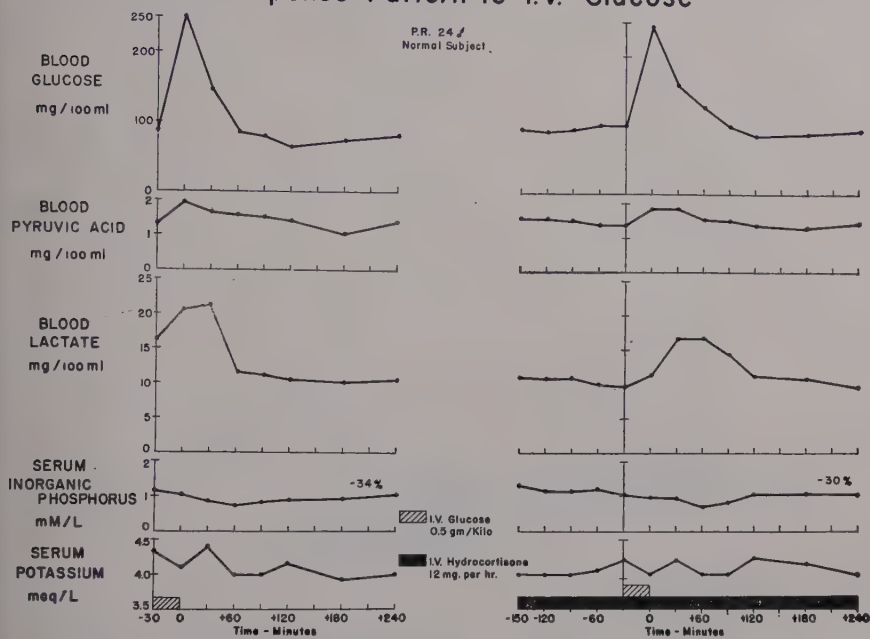


FIGURE 6. Intravenous glucose tolerance was unaltered by intravenous hydrocortisone infusion. The pyruvate response was greater, the serum phosphorus fall normal, and a slight early rise in potassium was noted.

tumor. Following a period of fasting and the development of hypoglycemic stupor, intravenous corticosterone in a dosage of 25 mg. per hour was compared with intravenous hydrocortisone in the same dosage. The infusion was given over an eight-hour period. Corticosterone caused a definite rise in blood glucose of 14 mg. per 100 ml., compared to a rise of 22 mg. with hydrocortisone. Pyruvic acid showed little change during the corticosterone infusion but rose sharply with hydrocortisone (FIGURE 9).

Since 1942, Ingle has been developing and emphasizing the interpretation that is now becoming more and more widely accepted, namely, that the adrenal cortex is necessary but not responsible for the metabolic changes after stress. A few metabolic responses thought to be regulated by the adrenal cortex can occur in its absence, although the presence of cortical hormone is required to support the response.<sup>26</sup> This concept of "permissive" action of adrenal steroids appears to be supported by our observations. Abbott *et al.*<sup>34</sup> have studied the pyruvate response during intravenous glucose tolerance tests in preoperative and postoperative patients. The pyruvate curves following major surgery were consistently higher than those seen in preoperative period, but this was not seen when less traumatic operations were performed.

As the result of numerous studies in man and animals, corticosterone has been shown to possess about one third to one half the glycogenic potency of hydrocortisone.<sup>7</sup> Therefore, the effect of 25 mg. per hour of corticosterone was

## The Effect of a Constant Infusion of Compound F on the Response to Intravenous Glucose

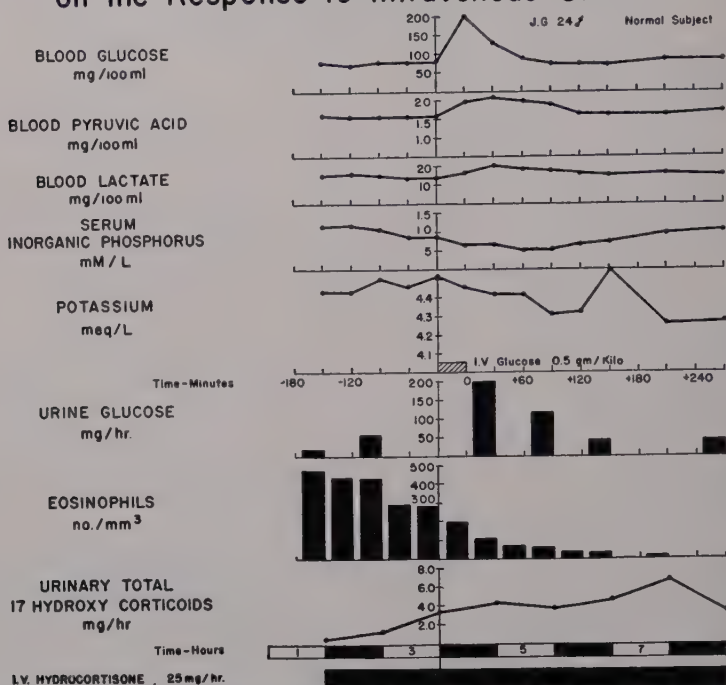


FIGURE 7. Hydrocortisone had no effect on glucose tolerance. The eosinopenic effect was marked. Pyruvate was elevated and prolonged.

compared in normal subjects to the effect of 12.5 mg. of hydrocortisone per hour (FIGURE 10). The control, the hydrocortisone, and the corticosterone glucose tolerance tests were normal and superimposable. The pyruvic acid response remained elevated longer with hydrocortisone than with corticosterone. The only effect noted with corticosterone was a delay in the maximum pyruvic acid response.

The unusual pyruvate response during intravenous corticoids led to a consideration of the effect of these steroids on fructose metabolism. The metabolism of fructose differs from glucose in both normal and diabetic subjects, because the metabolism of fructose in the diabetic is similar to that seen in normals, even in the absence of insulin.<sup>35</sup> Insulin acts on glucokinase, which converts glucose to glucose-6-phosphate, as proposed by Price, Cori, and Colowick,<sup>36</sup> whereas the phosphorylation of fructose is under the control of fructokinase, which is unaffected by the lack of insulin. Fructose phosphorylation, being independent of insulin, is presumably independent of the anti-insulin effect of adrenal steroids. Intravenous fructose (0.5 gm./kg. in a 10 per cent solution) was given during a constant infusion of hydrocortisone (12.5 mg. per hour (FIGURE 11). The normal subject handled fructose differently when given



# COMPARISON OF STEROID ALONE AND STEROID PLUS STRESS ON PYRUVIC ACID

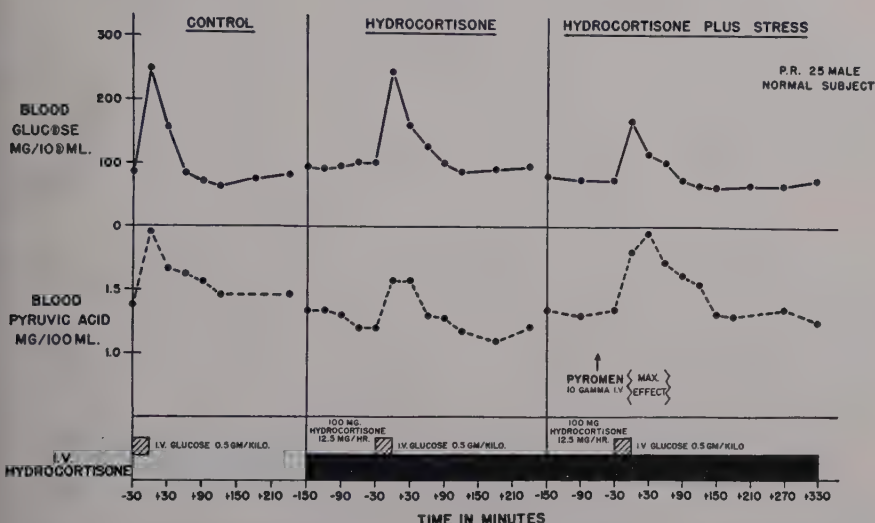


FIGURE 8. Pyromen accentuated the pyruvate response to hydrocortisone. The effect on glucose was slight.

## METABOLIC RESPONSE TO COMPOUNDS "B" AND "F" DURING STRESS OF HYPOGLYCEMIA

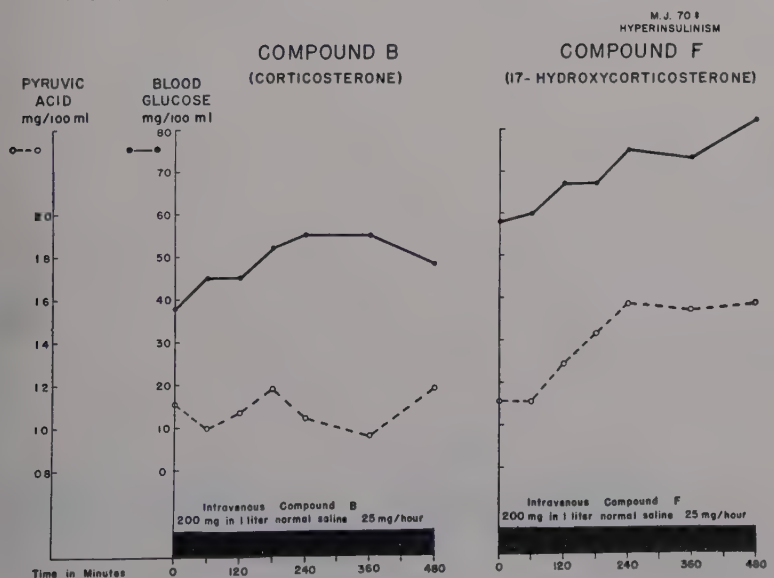


FIGURE 9. The pyruvate response to hypoglycemia, much more marked with hydrocortisone.

# COMPARISON OF PYRUVIC ACID RESPONSE DURING HYDROCORTISONE AND CORTICOSTERONE INFUSION

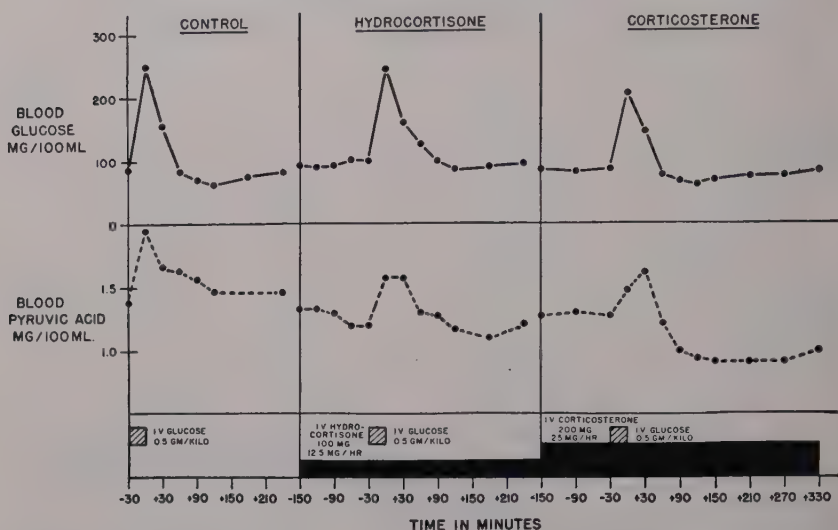


FIGURE 10. Both steroids had an effect on the pyruvate response when compared to the normal. Glucose tolerance remained unchanged.

## PYRUVIC ACID RESPONSE TO FRUCTOSE DURING A CONSTANT INFUSION OF COMPOUND F

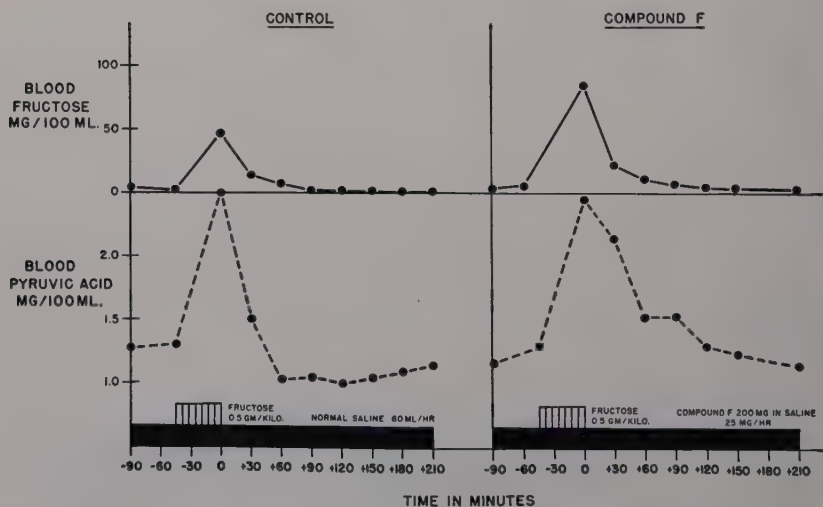


FIGURE 11. The pyruvate response after fructose was prolonged during hydrocortisone infusion.

hydrocortisone. The elevation of fructose was greater and the elevation of pyruvic acid, while not greater, was prolonged. This alteration in pyruvic acid may indicate that, with the rapid metabolism of fructose, it accumulates at the pyruvic acid level. When considered in light of previous observations of an elevated pyruvic acid following combined glucose and hydrocortisone administration, this suggests a decreased rate of removal of pyruvic acid. The fate of fructose during hydrocortisone infusion is similar to that reported by Drucker *et al.*,<sup>37</sup> who observed unimpaired fructose metabolism in patients undergoing operative stress.

### *Adrenal Steroids in Hypoglycemia*

The use of corticotropin and cortisone for the modification of blood glucose concentration has been justified ever since the work of Corey and Britton<sup>38</sup> and of Long<sup>39</sup> demonstrated that adrenal cortical extract, in excessive doses, exerted a diabetogenic effect in the normal animal. Corticotropin and adrenal glucocorticoids increase glycogen deposition in the liver, partly by increasing gluconeogenesis from amino acids, at the same time opposing the action of insulin peripherally. In adrenalectomized mice given insulin, the incidence of convulsions may be greatly reduced by administration of cortical extract or some 11-oxysteroids. This contrainsulin effect has been correlated with the accumulation of glycogen in the liver,<sup>40</sup> caused largely by the provision of extra glucose via gluconeogenesis. Long, Katzin, and Fry<sup>9</sup> found that the glycosuria of the partially depancreatized rat could be intensified by the administration of cortisone and other 11-oxysteroids, whereas 11-desoxycorticosterone acetate was ineffective. Ingle<sup>2, 3</sup> observed the production of glycosuria and hyperglycemia in rats by the administration of large amounts of 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone. It was also possible to duplicate these effects with corticotropin.<sup>41</sup> These steroids and pure corticotropin can be considered as effective diabetogenic agents.

The work of Houssay,<sup>42</sup> Evans,<sup>43</sup> Baumann,<sup>44</sup> and Young<sup>45</sup> indicated that continued administration of extracts of anterior lobe of the pituitary produced glycosuria and hyperglycemia in the normal animal. The use of corticotropin in idiopathic hypoglycemia has been reported by McQuarrie *et al.*<sup>46</sup> After an initial course of corticotropin, the patients were maintained satisfactorily on only two injections of corticotropin per week. The beneficial results obtained, the use of long-term therapy, and the remarkably small quantities of corticotropin required are worthy of note. Baker *et al.*<sup>47</sup> observed stimulation of the pancreatic islet tissue of rats given large doses of corticotropin. Hypertrophy, hyperplasia, and degranulation of the beta cells were observed. A somatotrophic preparation given to rats did not affect significantly the size of the islets of Langerhans nor the cytology of the beta cells.

The other "diabetogenic" factor of anterior pituitary extracts, in addition to corticotropin, is believed to be growth hormone. Growth hormone: (1) depresses the respiratory quotient of fasted hypophysectomized or fed normal rats; (2) prevents the severe loss of muscle glycogen observed when hypophysectomized animals are fasted; and (3) depresses the glucose uptake of the iso-

lated diaphragm. In addition, when given to partially depancreatized rats or to animals made diabetic with alloxan, it causes a marked exacerbation of the diabetes. The normal rat and guinea pig are relatively resistant to the diabetogenic action of growth hormone, which will produce permanent diabetes in dogs and cats, undoubtedly due to irreversible damage to the islets of Langerhans.<sup>48</sup> Cortisone alone has a slight diabetogenic action in cats but, when combined with small doses of growth hormone, has a synergistic effect and marked hyperglycemia and glycosuria develop.<sup>49</sup>

*Hypoglycemia due to hyperinsulinism.* Conn<sup>50</sup> has established an etiological classification of spontaneous hypoglycemia—functional hyperinsulinism, organic hyperinsulinism, and hepatogenic hypoglycemia. Of the organic lesions, hyperinsulinism due to pancreatic islet cell adenoma, carcinoma, or hyperplasia, and relative hyperinsulinism due to adrenal cortical hypofunction are of importance to this discussion.

Several reports describing the use of corticotropin or cortisone in alleviating hypoglycemia due to an islet cell tumor have appeared since McQuarrie's earlier work treating spontaneous hypoglycemia of infants with corticotropin. G. M. Brown<sup>51</sup> was disappointed with the effects of corticotropin given to one patient with an islet cell adenoma. It should be stated, however, that this patient had little or no eosinophil response, although receiving 25 mg. every six hours for three days. Smith and Cochran<sup>52</sup> found both corticotropin and cortisone useful. H. Brown, Hargreaves, and Tyler<sup>53</sup> were impressed with the effectiveness of corticotropin compared to cortisone in regard to both relief of hypoglycemic symptoms and duration of action. Mason<sup>54</sup> has treated successfully with oral cortisone a patient who had a recurrence of hyperinsulinism due to liver metastasis from a previously removed malignant islet-cell tumor. Osnes and Thorsen<sup>55</sup> treated with corticotropin and cortisone four patients having insulinomas, with abolition of hypoglycemic attacks. While awaiting surgery in a seven-year-old child with an islet-cell adenoma, Roxburgh<sup>56</sup> observed a normal glucose tolerance curve after two weeks of corticotropin treatment. De Peyster and Gilchrist<sup>57</sup> reported three cases of spontaneous hypoglycemia, ultimately treated by surgery, who obtained preoperative and interim responses to dietary, alloxan, corticotropin, and cortisone therapy.

Black, in collaboration with Young<sup>58</sup> gave two trials of growth hormone therapy to a patient with hyperinsulinism due to an islet-cell tumor. The observed effects of growth hormone were as follows: a substantial reduction in the carbohydrate intake required to prevent hypoglycemia (first trial); a slight over-all rise in the blood sugar level (first trial, 60 mg. dose) or a well-marked but transient rise when glucose supplements were standardized (second trial, 100 mg. dose); and loss of weight in spite of increased caloric intake. Growth hormone had a negligible short-term effect on the blood-sugar level, as shown by its failure to relieve an attack of hypoglycemia.

The fact that, under certain circumstances, growth hormone exhibits an hypoglycemic rather than hyperglycemic action may be due to a stimulation of insulin secretion. Thus, it may require longer trials of growth hormone before determining whether it will cause either direct islet exhaustion or exhaustion due to a continuing supply of carbohydrate precursors.



# EFFECT OF ADRENAL CORTICAL STEROIDS ON SPONTANEOUS HYPOGLYCEMIA

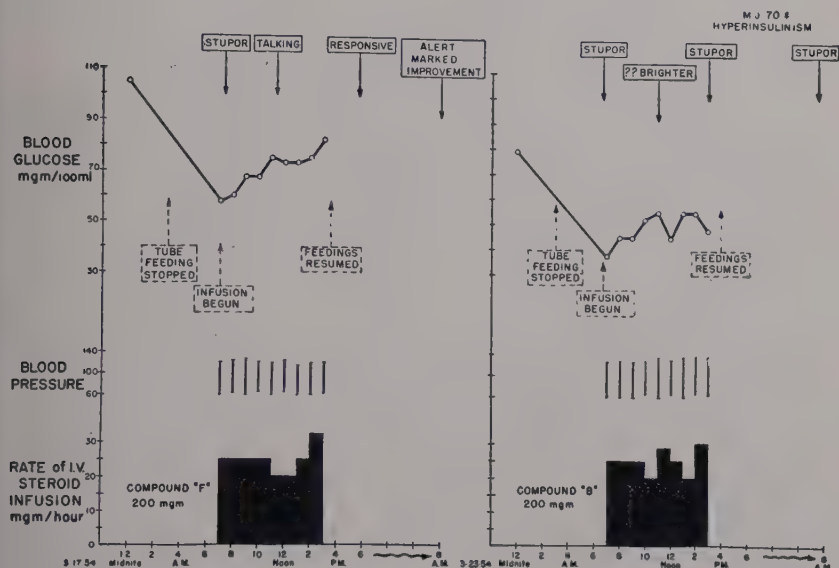


FIGURE 12. Intravenous hydrocortisone, more effective than intravenous corticosterone in hypoglycemia.

Prior to surgery, studies were performed using intravenous hydrocortisone and corticosterone in a 70-year-old woman with hyperinsulinism. The triad of Whipple's was present: the blood sugar having fallen to between 17 and 25 mg. per 100 ml. on numerous occasions after fasting, glucose administration relieved insulin shock, and all other possible factors that might contribute to the production of hypoglycemia were eliminated, such as pituitary-adrenal cortical, hepatic, renal, and central nervous system factors. The patient would lapse into a stuporous state in six or seven hours after discontinuing tube feedings. During one episode of stupor, an infusion of 200 mg. of hydrocortisone (Cortef) was given over an eight-hour period (FIGURE 12). The blood sugar gradually rose from 58 mg. to 68 mg. in two hours, and after four hours was 72 mg. The patient began to talk and become more responsive. Twenty-four hours later, the patient showed greater clinical improvement than had been noticed on many other occasions when the blood sugar was normal or above.

An infusion of corticosterone, 200 mg. intravenously over an eight-hour period, was given during another episode of stupor. There was a rise in the blood sugar from 38 mg. to 52 mg. with questionable improvement in her mental state. Unlike hydrocortisone, the improvement was not maintained over the next 24 hours, and she was again in stupor. Corticosterone produced effects on carbohydrate metabolism qualitatively, similar to that produced by hydrocortisone, but much less intense. Corticosterone does appear to exert an anti-insulin effect.

It was concluded that hydrocortisone intravenously was more effective than

corticosterone in relieving hypoglycemic symptoms. This is not unexpected in view of the known superiority of hydrocortisone in terms of carbohydrate activity. It also appears that hydrocortisone effectively raises the tolerance for hypoglycemia without greatly modifying the blood sugar. The duration of this effect is also greater with hydrocortisone than with corticosterone.

Intravenous hydrocortisone is an acceptable and advantageous method of dealing with the effects of hypoglycemia. In acute hypoglycemia, it possesses the advantages of a rapid effect and a relatively prolonged action, and it avoids the uncertainty of steroid absorption which accompanies other modes of steroid administration. Intravenous hydrocortisone, however, does not possess the sustaining effect of intramuscularly administered cortisone-like steroids or repeated oral doses. Its specificity as an insulin antagonist promises to make it a more useful agent in an emergency. McQuarrie<sup>59</sup> has observed instances of hypoglycemia that are refractory to corticotropin. In no sense can it be concluded that steroids are an acceptable method of treating insulin adenoma, except where surgery is impossible. The greatest usefulness of steroids is likely to be in the management of acute hypoglycemia, preoperatively and where prolonged medical management is necessary.

The changes in pyruvic acid observed with hydrocortisone and corticosterone have already been discussed (FIGURE 9).

Blood alcohol measurements were obtained in one patient during the infusion of 200 mg. of a 20 per cent alcoholic solution of hydrocortisone in 500 ml. of normal saline, administered over an eight-hour period. The blood alcohol rose very slightly after four hours to 5.8 mg. and, at the end of eight hours, was only 19.6 mg., which is well below toxic levels (FIGURE 13).

The observations of Griffiths<sup>60</sup> that intravenous hexamethonium powerfully potentiates the action of parenteral insulin in anesthetized man, both diabetic



FIGURE 13. Blood sugar and blood alcohol responses during intravenous hydrocortisone.

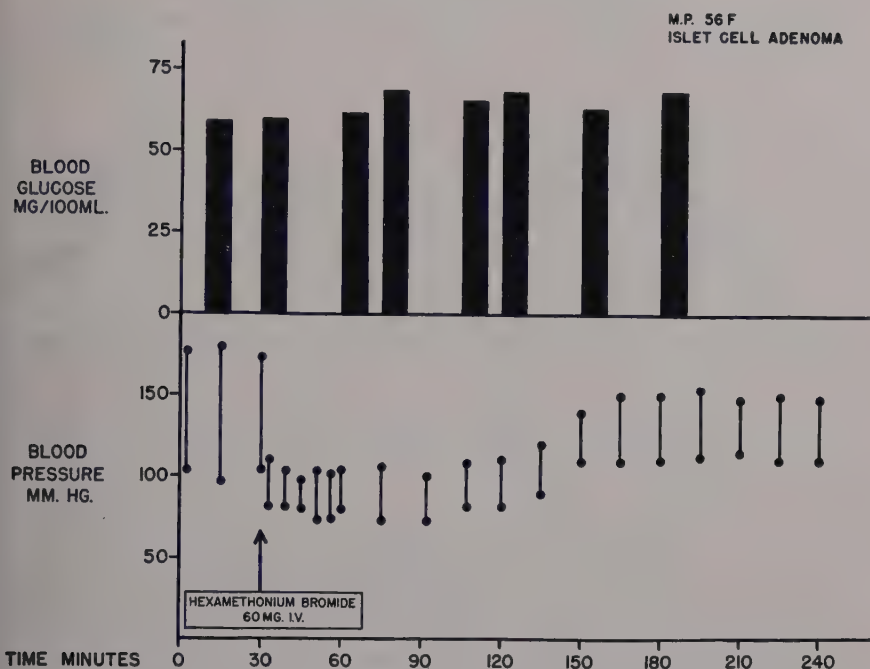


FIGURE 14. Nonpotentiating effect of hexamethonium in spontaneous hypoglycemia.

and nondiabetic, stimulated an investigation of this compound in spontaneous hypoglycemia. A patient with classical features and findings of hypoglycemia due to islet-cell adenoma was given intravenous hexamethonium (60 mg.) as a test prior to surgical exploration, at which time an islet-cell adenoma was removed. Following a period of fasting of eight to ten hours, this patient regularly manifested hypoglycemia. Following such a fast period, a control blood sugar was drawn and basal blood pressures were obtained (FIGURE 14). The hexamethonium was administered rapidly with a prompt marked reduction in blood pressure. No measurable effect on the blood sugar was observed over a four-hour period, and the blood pressure gradually returned toward control levels. Hexamethonium does not potentiate hypoglycemia in man. It would appear that, in the unanesthetized state, the autonomic-block induced by hexamethonium is insufficient to prevent the compensatory glycogenolysis which develops through the release of adrenal medullary hormone. Other explanations to be considered are that the dosage of hexamethonium was not sufficient to prevent adrenaline release, or that factors other than adrenaline are responsible for the return of blood glucose to normal following insulin hypoglycemia.

*Hypoglycemia of Addison's disease.* The most striking alteration in carbohydrate metabolism in adrenal-deficient subjects is the tendency to develop hypoglycemia during fasting. It has long been known that hypoglycemia occurs with great frequency in adrenalectomized animals. The case with

which hypoglycemia signs and symptoms can be induced by prolonged fasting, by treatment with insulin or phlorhizin, by feeding a diet adequate in calories but low in carbohydrate, and by administration of standard glucose tolerance test has been demonstrated.<sup>61</sup>

There is a rapid depletion of liver glycogen and, later, a gradual depletion of muscle glycogen. In rats, the hypoglycemia is accompanied by a diminished rate of urinary nitrogen excretion. This would indicate that, in the adrenalectomized animal, the development of hypoglycemia during fasting is due in part to the decreased rate of gluconeogenesis from the tissue proteins. A quantitative study of the effects of injecting adrenal cortical extracts and steroids of the cortisone type into fasting adrenalectomized rats revealed a significant elevation of blood glucose and a tenfold to twentyfold increase in liver glycogen without any change in muscle glycogen.<sup>9</sup> Consequently, there must have occurred an accelerated rate of tissue protein catabolism, as confirmed by the observation that there is an increased urinary nitrogen excretion during the hormone injection which accounts for some of the new carbohydrate found in the body fluids and the liver.

The choice of 17-hydroxycorticosterone as, possibly, a more effective antagonist of insulin than cortisone is based on the observations of Thorn<sup>62</sup> that this compound is more effective than cortisone in eliciting eosinopenia in man and those of Conn,<sup>63</sup> who found 17-hydroxycorticosterone is the more potent of the two compounds in eliciting metabolic changes in man. Baird and Munro<sup>64</sup> compared the effect of hydrocortisone and cortisone in a patient with coexisting diabetes mellitus and Addison's disease, and found hydrocortisone more diabetogenic than cortisone in doses of 5 and 10 mg. but, at higher doses (25 mg.), this difference was no longer apparent. Using the muscle-performance of adrenalectomized rats, Ingle, Nezamis, and Morley<sup>25</sup> found 17-hydroxycorticosterone to have about twice the biologic potency of cortisone. This confirmed previous observations that 17-hydroxycorticosterone had greater biologic activity than cortisone by the Ingle work test and by the liver glycogen deposition test.<sup>7</sup>

The most complete evaluation of the capacity of 11- and 11,17 oxysteroids to increase the levels of carbohydrate in patients with Addison's disease has been carried out by Thorn and Forsham.<sup>65</sup> They noted the ability of compounds A, B, E, and F to increase carbohydrate levels. Compound A, 11-dehydrocorticosterone, caused no significant increase in the fasting blood sugar in Addisonians, but enabled them to withstand a 24-hour fast without developing hypoglycemia. Compound E, 11-dehydro-17-OH-hydroxycorticosterone, also prevented hypoglycemia, but also caused an elevation in fasting blood sugar. Similar observations in Addison's disease using compound A and compound E were made by Sprague.<sup>66</sup>

In FIGURE 15 are shown typical hypoglycemic patterns which may be observed in Addison's disease.<sup>67</sup> Two patients had a fall in blood sugar following a prolonged fast and developed hypoglycemia of a severe degree. Two other patients subjected to the same procedure of fasting also developed hypoglycemic symptoms, but without any measurable change in blood sugar! The



# VARIATIONS IN BLOOD SUGAR OBSERVED DURING PROLONGED FAST IN SEVERE ADDISON'S DISEASE

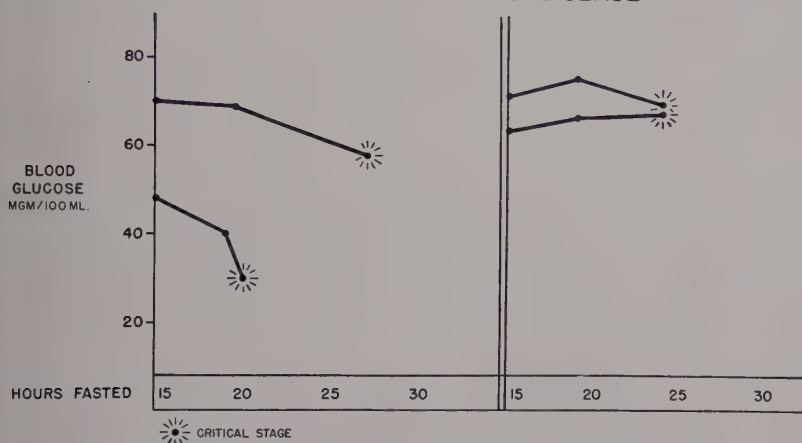


FIGURE 15. Two different patterns of hypoglycemic response in Addison's disease are compared (after Thorn *et al.*<sup>67</sup>).

threshold for developing hypoglycemia is significantly altered in adrenal cortical insufficiency (FIGURES 3 and 4).

Intravenous hydrocortisone was given to a patient with Addison's disease following a prolonged fast (FIGURE 16). The fast had no effect on blood sugar, but the patient had severe symptoms including profound weakness and then collapse. At this stage, intravenous hydrocortisone was given at the rate of 25 mg. per hour. There was a prompt improvement and, after one hour, the patient felt markedly improved although the blood sugar did not change significantly. After four hours, the patient had completely recovered, and the dosage rate of the hydrocortisone was doubled to 50 mg. per hour. At this dosage, the blood sugar began to rise appreciably. After two hours, with the addition of a small meal, the blood sugar increased even more. Following the discontinuance of hydrocortisone, the blood sugar gradually returned to normal levels. The weakness associated with adrenal deficiency may be associated with hypoglycemia, but a continuous intravenous infusion of glucose alone improves only slightly the ability for muscle work in adrenalectomized animals.<sup>68</sup>

It may be difficult, as has been pointed out by Thorn,<sup>69</sup> to determine the efficacy of adrenal cortical steroids in treating the hypoglycemia of Addison's disease because, although the glycogen-storing capacity of the steroid may be marked, these patients are unable to mobilize these stores rapidly due to a coexisting adrenal medullary hormone deficiency. The change in the blood sugar level may be only minimal, but the clinical improvement is marked. This has been borne out also in our studies using intravenous adrenal steroids.

The concept of "effective" blood sugar levels; *viz.*, the ability of blood glucose to enter into cells proposed by Bates and Mayer,<sup>70</sup> is suggested by the dramatic efficacy of adrenal steroids in the hypoglycemia of adrenal insufficiency. These

# EFFECT OF INTRAVENOUS HYDROCORTISONE IN ADDISON'S DISEASE DURING A PROLONGED FAST

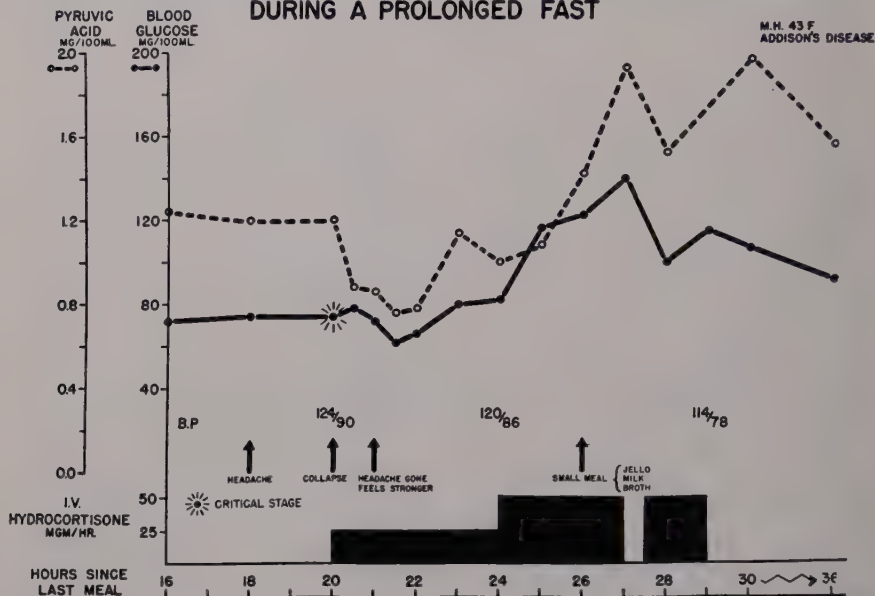


FIGURE 16. Marked symptomatic relief after hydrocortisone without a rise in blood sugar. The pyruvic acid response was marked.

same authors consider that the uncontrolled diabetic has a hyperglycemia, but a "metabolic hypoglycemia" which, according to the glucostatic theory, controls the hunger feeling in such patients. It might be postulated that the Addisonian, under fasting conditions, has a hypoglycemia and a "metabolic hypoglycemia." With the administration of adrenal steroids, however, the "metabolic hypoglycemia" becomes a "metabolic normoglycemia" that relieves the hypoglycemic symptoms.

It is becoming increasingly apparent that the level of the blood sugar is not always a reliable index of the presence of the hypoglycemic state. Duras<sup>71</sup> and, later, Webster and Blades<sup>72</sup> have emphasized the lack of correlation between the blood sugar level and clinical attacks of hypoglycemia in functioning islet-cell tumors. The beneficial effects of intravenous hydrocortisone in the hypoglycemic attacks of our patient with hyperinsulinism and the patients with hypoglycemia due to adrenal insufficiency emphasize that the relief of clinical hypoglycemia does not require concomitantly a marked increase in the blood sugar.

Conn, Louis, and Fajans<sup>73</sup> have suggested that 17-hydroxycorticosterone may possibly be the only natural secretory product of the adrenal cortices. The metabolic effects of hydrocortisone acetate and free hydrocortisone, while qualitatively similar, appear to be, quantitatively, slightly different. Studies of the efficacy of the nonesterified compounds administered orally have shown that the antirheumatic effect of hydrocortisone is greater than cortisone per

unit weight of the steroid.<sup>74</sup> Ingle<sup>75</sup> used constant subcutaneous infusions of nonesterified steroids in normal rats and found that, while every evidence of hypercorticalism could be produced by either cortisone or hydrocortisone, the latter was twice as effective. The speed of action of free hydrocortisone is greater, because it lacks esterification, thus giving it a more intense metabolic and therapeutic action. This is a particularly desirable feature in situations requiring immediate therapeutic levels of adrenal hormone.

In our limited experience, we find it difficult to agree entirely with the suggestion that intravenous free hydrocortisone will provide all the metabolic effects of whole adrenal cortex extract.<sup>76</sup> While it has been extremely effective in combatting the hypoglycemia of hyperinsulinism due to islet overproduction, and in relieving the hypoglycemic symptoms of acute adrenal insufficiency, we have found it inadequate in maintaining Addison's disease patients during major surgical procedures. Such patients do not always maintain an adequate blood pressure until adrenal extract is given. There is also a great deal of experimental evidence obtained in animals which suggests that, thus far, none of the isolated individual steroids given separately or in combination completely sustain the adrenalectomized animal,<sup>77</sup> but that adrenal cortex extracts will. Extracts of adrenal glands, for example, elicit a greater peak response than does 17-hydroxycorticosterone, and the "amorphous fraction" of such extracts has been shown to have greater biological activity per unit weight than does any known adrenal steroid.

#### *Steroid Diabetes versus Pancreatic Diabetes*

The term steroid diabetes was introduced by Ingle to describe the type of diabetes induced by the administration of compounds B, E, F, and corticotropin in rats.<sup>78</sup> The diabetes accompanying pheochromocytoma may also represent an instance of steroid diabetes due to excessive stimulation of the pituitary-adrenal axis by epinephrine.

The diabetes induced by adrenal steroids appears to differ somewhat from pancreatic diabetes, in that animals are enormously resistant to insulin and such associated effects as diminution in glucose tolerance, and ketosis. Loss of water, salts, and nitrogen are often not commensurate with the degree of glycosuria. The concept that the primary action of cortisone and related compounds is to stimulate gluconeogenesis from protein does not fully explain the extent of glycosuria or the insulin resistance that can be induced by these compounds.<sup>78</sup> It seems necessary to assume that these hormones either stimulate gluconeogenesis from fat, or that they inhibit some phase of carbohydrate utilization (oxidation, storage as glycogen, or conversion to fat). The observations described above on pyruvic acid metabolism favor the concept of inhibition.

The sensitivity of animals to the production of diabetes with steroids is variable. Rats develop steroid diabetes only when they are force-fed,<sup>2, 78</sup> while guinea pigs are extremely susceptible to steroids and develop a diabetes essentially identical to that accompanying hypercorticalism in man.<sup>79</sup>

The incidence of diabetes, either frank or latent, is high in Cushing's syn-

drome, due either to a hyperfunctioning tumor or to adrenal cortical hyperplasia. In the majority of cases, the diabetes is mild and may require a glucose tolerance test for its demonstration. A few cases are associated with a high insulin requirement. Plotz *et al.*<sup>13</sup> reported a series of 33 patients with Cushing's syndrome, of whom 31 exhibited diabetic glucose tolerance curves, but only five had frank diabetes. Sprague, Mason, and Power<sup>80</sup> found diminished carbohydrate tolerance in 11 consecutive patients with Cushing's syndrome. Generally, steroid diabetes is relatively mild and relatively insulin insensitive. It is usually temporary, disappearing with the correction of the steroid excess.

Many workers have commented on the clinical and laboratory differences that obtain between pancreatic diabetes and steroid diabetes. These differences include the dissociation between blood glucose and intensity of glycosuria, the difference in insulin sensitivity, and the reversibility of steroid diabetes upon correction of hypercorticalism. Bookman<sup>81</sup> cites five cases in which diabetes developed during cortisone or corticotropin therapy. The diabetes disappeared in four cases. Lasting diabetes may have been caused by corticotropin in Bishop's case.<sup>82</sup>

An increasing number of studies in the last three years have suggested that other differences exist between steroid diabetes and pancreatic diabetes that involve the intermediary metabolites of glucose rather than glucose alone. For example, it is becoming more fully appreciated that alterations in blood glucose may be an unreliable index if used as the sole criterion of the effect of steroids on carbohydrate metabolism. The studies already included in this paper, as well as others, suggest that pyruvic acid, potassium, and phosphorus may be altered in a manner quite unlike that ordinarily seen in pancreatic diabetes.

*Pyruvate metabolism in steroid diabetes.* Himwich and Himwich<sup>83</sup> observed that diabetic men and dogs formed pyruvate normally after muscular exercise, but that they did not do so in response to ingested glucose. The subject was studied further by Horwitt, Hills, and Kreisler,<sup>84</sup> who noted that patients with moderately severe diabetes, when not receiving insulin, exhibited a rise in both blood lactate and blood pyruvate when glucose was ingested, but that these elevations were retarded. Insulin administration restored the elevation of lactate and pyruvate levels. Bueding<sup>15</sup> observed in normal individuals a significant rise in lactic and pyruvic acids after glucose administration. In patients with diabetes mellitus, this rise was either absent or delayed, but the concentration of these substances could be increased by administering insulin.

Our investigations in pyruvic acid metabolism have confirmed the observations of Bueding *et al.* in patients with diabetes mellitus. In Cushing's syndrome, increases in fasting blood levels of pyruvic acid have been obtained. Increases also occur in patients receiving prolonged corticotropin or 11-oxy steroid therapy. Of particular interest was the finding of an elevated pyruvic acid in these patients without any other evidence of an abnormal glucose metabolism by either the fasting blood sugar or glucose tolerance.

Hills<sup>85</sup> studied 34 mild and borderline cases of diabetes mellitus and 11 subjects with the clinical diagnosis of Cushing's syndrome. In most of the subjects with glucose tolerance curves which suggested diabetes mellitus but were not conclusively diagnostic, the levels of lactate and pyruvate also proved to



be equivocal. In 5 of the 11 cases of Cushing's disease, the glucose tolerance curves were normal. In these, the levels of lactate and pyruvate also were essentially what would be expected in the normal. In six of the cases with Cushing's disease, however, the glucose tolerance curves were frankly diabetic and, in four of them, the levels of lactate and pyruvate were elevated before glucose was given for a glucose tolerance test, and even more elevated after giving glucose.

In summary, an increasing amount of evidence suggests that adrenal cortical hormones exert a definite influence on pyruvic acid metabolism. The finding of elevated fasting blood pyruvic acid levels in association with 11-oxysteroid excesses, and the elevation of pyruvic acid, following glucose, contrasts strikingly with the lack of elevation of pyruvic acid in diabetes mellitus.

*Potassium metabolism in steroid diabetes.* Many observations point to a strong connection between potassium and carbohydrate metabolism. Insulin will lower the serum potassium, as will other factors promoting hepatic glycolysis. In a case of spontaneous hypoglycemia, McQuarrie *et al.*<sup>86</sup> observed a rise in blood sugar with partial relief of symptoms following administration of potassium chloride.

An increased renal clearance of potassium and eventual potassium depletion is a prominent metabolic effect of the group of cortisone-like steroids. Cushing's syndrome due to adrenal cortical tumor, to adrenal hyperplasia, or secondary to prolonged corticotropin or cortisone therapy not infrequently develop hypokalemic, hypochloremic alkalosis. Kinsell *et al.* noted that the administration of potassium to diabetic patients who were receiving corticotropin or cortisone appeared to decrease or prevent insulin resistance.<sup>87</sup> They postulated that the diabetogenic effect of cortical steroids and the steroid-induced insulin resistance might be due, in part, to the potassium depletion associated with these steroids. In one female patient with mild diabetes, who was also receiving cortisone but no insulin, the administration of large amounts of potassium reduced insulin resistance.<sup>88</sup>

Wilson *et al.*<sup>89, 10</sup> have reported a fall in serum potassium concentration during the intravenous glucose tolerance test in diabetics, as in nondiabetics receiving corticotropin treatment. Similar findings have been observed in patients with Cushing's syndrome in spite of the presence of a normal glucose tolerance test (TABLE 2). This represents an additional metabolic phenomenon similar to others seen in diabetes, even though hyperglycemia and an abnormal glucose tolerance test may be absent.

TABLE 2  
CHANGES IN SERUM POTASSIUM CONCENTRATIONS DURING AN INTRAVENOUS GLUCOSE TOLERANCE TEST

Clinical state	Fall in serum potassium mEq. per liter
Normal.....	-0.17
Diabetes mellitus.....	-0.56
ACTH.....	-0.90
Cushing's syndrome.....	-0.70

After Wilson *et al.*<sup>10</sup>

During our studies with intravenous hydrocortisone, a slight rise in serum potassium was observed after two hours of the infusion at rates of 12 mg. and 25 mg. of hydrocortisone per hour (FIGURES 6 and 7). Recently, Knight, Kornfeld, Glaser, and Bondy<sup>90</sup> also noted an increase in serum potassium with intravenous hydrocortisone given in doses of 50 mg. or 100 mg. over a four-hour period. Failure to observe greater and more sustained rises in the serum potassium of our subjects after intravenous hydrocortisone may be explained by the institution of an intravenous glucose tolerance, beginning with the third hour of intravenous hydrocortisone.

*Phosphorus metabolism in steroid diabetes.* Forsham and Thorn<sup>91</sup> observed with corticotropin and cortisone that the glucose tolerance was definitely elevated, but that the fall in serum inorganic phosphorus persisted. This is in contrast to the poor phosphorus fall observed in association with the hyperglycemic type of blood sugar curve of diabetes mellitus. Hayes and Brandt<sup>92</sup> found a normal serum phosphate fall in postoperative patients despite impaired glucose tolerance. Our studies indicate that, with the technique of performing a glucose tolerance test during a constant infusion of hydrocortisone, there is no interference with the fall in serum phosphorus (FIGURE 6). This lack of effect on phosphorus is also observed in steroid diabetes, although the glucose tolerance is abnormal. More recently, Kupperman *et al.*<sup>93</sup> found that the oral glucose tolerance test was abnormal when performed during an intravenous infusion of hydrocortisone, but that the blood phosphorus in these patients showed a fall indicative of peripheral utilization of glucose.

Before drawing too definite conclusions regarding the values of the differences in serum phosphorus response as they occur in steroid diabetes and pancreatic diabetes, it will be necessary to have more evidence regarding the actual fate of the phosphorus in steroid diabetes. For example, it is known that the urinary excretion of phosphorus is increased in the presence of cortisone. Therefore, the fall in serum levels accompanying steroid diabetes may not be due solely, if at all, to participation of phosphate in glucose utilization.

A comparison of some of the metabolic responses to glucose as observed in steroid diabetes and diabetes mellitus in man has been summarized in TABLE 3. To these can be added the low urinary steroid excretion in diabetes mellitus as compared to steroid diabetes and the disparity that exists between the level of blood sugar and the significant increase in glycosuria which accompanies steroid administration. Recently, Bastenie *et al.*<sup>94</sup> in Belgium observed a paradoxical effect with cortisone in three of eight cases of diabetes in bearded women. Not only did this treatment fail to increase glucose intolerance, glycosuria, or insulin requirement, but it actually improved these characteristics.

TABLE 3  
A COMPARISON OF SOME METABOLIC RESPONSES TO GLUCOSE IN DIABETIC STATES

	"Steroid" diabetes	Diabetes mellitus
Glucose tolerance.....	Normal or decreased	Decreased
Blood pyruvic acid .....	Elevated	No change
Serum potassium.....	Decreased	Decreased
Serum phosphorus .....	Decreased	No change

*Summary*

The advent of intravenous preparations of pure crystalline adrenal steroid preparations has made it possible to investigate more precisely the role of adrenal steroids in carbohydrate metabolism in man. The results obtained thus far suggest that certain intermediates of carbohydrate, particularly pyruvic acid, may be altered significantly with or without marked alterations in glucose. The elevations of pyruvic acid observed after glucose and fructose, and also in stress situations due to illness or stressors have been described. It is suggested that the increases in pyruvic acid under these conditions is due to an interference with its removal during carbohydrate utilization.

Intravenous hydrocortisone has been found useful in treating hypoglycemic symptoms due to hyperinsulinism from an islet-cell tumor and in the hypoglycemic manifestations accompanying adrenal cortical insufficiency. The striking clinical benefit obtained without accompanying marked changes in blood glucose is worthy of particular note.

Steroid diabetes has been shown to possess certain similarities to, and numerous differences from, diabetes mellitus. The changes in pyruvic acid, potassium, and phosphorus metabolism which characterize and distinguish steroid diabetes from diabetes mellitus are emphasized.

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*Bibliography*

1. INGLE, D. J. 1940. Diabetogenic effect of some cortin-like compounds. *Proc. Soc. Exptl. Biol. Med.* **44**: 176-177.
2. INGLE, D. J. 1941. Production of glycosuria in normal rat by means of 17-hydroxy-11-dehydrocorticosterone. *Endocrinology*. **29**: 649-652.
3. INGLE, D. J., R. SHEPPARD, E. A. OBERLE & M. H. KUIZENG. 1946. A comparison of the acute effects of corticosterone and 17-hydroxycorticosterone on body weight and the urinary excretion of sodium, chloride, potassium, nitrogen and glucose in the normal rat. *Endocrinology*. **39**: 52-57.
4. INGLE, D. J., C. H. LI & H. M. EVANS. 1946. The effect of adrenocorticotrophic hormone on the urinary excretion of sodium, chloride, potassium, nitrogen and glucose in normal rats. *Endocrinology*. **39**: 32-42.
5. FORSHAM, P. H., G. W. THORN, G. E. BERGNER & K. EMERSON, JR. 1946. Metabolic changes induced by synthetic 11-dehydrocorticosterone. *Am. J. Med.* **1**: 105-134.
6. PERERA, G. A., D. W. BLOOD & K. H. REINHOLD. 1946. 11-dehydrocorticosterone. Its effects on hypoadrenalism in man. *Am. J. Med.* **1**: 135-143.
7. PABST, M. L., R. SHEPPARD & M. H. KUIZENG. 1947. Comparison of liver-glycogen deposition and work performance tests for the bio-assay of adrenal cortex hormones. *Endocrinology*. **41**: 55-65.
8. LONG, C. N. H. & F. D. W. LUKENS. 1936. The effects of adrenalectomy and hypophysectomy upon experimental diabetes in the cat. *J. Exptl. Med.* **63**: 465-490.
9. LONG, C. N. H., B. KATZIN & E. G. FRY. 1940. The adrenal cortex and carbohydrate metabolism. *Endocrinology*. **26**: 309-344.
10. WILSON, D. R., T. F. FRAWLEY, P. H. FORSHAM & G. W. THORN. 1950. The functional relationship between the pancreatic islets and the adrenal cortex in man. *Proc. Am. Diabetes. Assoc.* **10**: 25-34.
11. CONN, J. W., L. H. LOUIS & M. W. JOHNSTON. 1948. Studies upon mechanisms in-

- involved in the induction with adrenocorticotrophic hormone of temporary diabetes mellitus in man. *Proc. Am. Diabetes. Assoc.* **8**: 215-239.
12. FAJANS, S. S. & J. W. CONN. 1954. An approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone. *Diabetes*. **3**: 296-304.
  13. PLOTZ, C. M., A. I. KNOWLTON & C. RAGAN. 1952. The natural history of Cushing's syndrome. *Am. J. Med.* **13**: 597-614.
  14. FRIEDEMANN, T. E. & G. E. HAUGEN. 1943. Pyruvic acid. II. The determination of keto acids in blood and urine. *J. Biol. Chem.* **147**: 415-442.
  15. BUEDING, E., H. WORTIS & H. D. FEIN. 1942. Pyruvic acid metabolism in diabetes mellitus. *Am. J. Med. Sci.* **204**: 838-845.
  16. LEWIS, R. A., D. KUHLMAN, C. DELBUE, G. F. KOEPF & G. W. THORN. 1940. The effect of the adrenal cortex on carbohydrate metabolism. *Endocrinology*. **27**: 971-982.
  17. KERPPOLA, W. 1953. Accumulation of pyruvic acid in the blood during treatment with cortisone or ACTH, and in Cushing's syndrome. *Acta Med. Scand.* **145**: 357-360.
  18. GITELSON, S. 1954. The effect of ACTH and cortisone on the blood pyruvic acid level. *Acta Endocrinol.* **15**: 225-235.
  19. LÖVGREN, O. 1952. The effect of cortisone and ACTH treatment on certain carbohydrate metabolites. *Acta Endocrinol.* **9**: 421-427.
  20. KEYES, G. H. & V. C. KELLEY. 1949. Glycogenic effect of adrenal cortical extract. *Am. J. Physiol.* **158**: 351-357.
  21. FIELD, J. B. & A. MARBLE. 1951. Diminished adrenocortical function in diabetes as shown in eosinophil response to stress of surgery. *Proc. Soc. Exptl. Biol. Med.* **77**: 195-198.
  22. TALBOT, N. B., M. S. WOOD, J. WORCESTER, E. CHRISTO, A. M. CAMPBELL & A. S. ZYGUNTOWICZ. 1951. Further observations on the excretion of water-soluble corticosteroids by normal subjects. *J. Clin. Endocrinol.* **11**: 1224-1236.
  23. FORBES, A. P., E. C. DONALDSON, E. C. REIFENSTEIN, JR. & F. ALBRIGHT. 1947. The effect of trauma and disease on the urinary 17-ketosteroid excretion in man. *J. Clin. Endocrinol.* **7**: 264-288.
  24. MILLER, S. & H. L. MASON. 1945. The excretion of 17-ketosteroids by diabetics. *J. Clin. Endocrinol.* **5**: 220-225.
  25. INGLE, D. J., J. E. NEZAMIS & E. H. MORLEY. 1951. Work performance of adrenalectomized rats given cortisone and 17-hydroxycorticosterone by continuous intravenous injection. *Proc. Soc. Exptl. Biol. Med.* **78**: 79-81.
  26. INGLE, D. J., R. C. MEEKS & K. E. THOMAS. 1951. The effect of fractures upon urinary electrolytes in non-adrenalectomized rats and in adrenalectomized rats treated with adrenal cortical extract. *Endocrinology*. **49**: 703-708.
  27. FRAWLEY, T. F., J. J. GARRETT & P. J. ROSCH. 1954. Metabolic adjustments during stress: The effect of intravenous glucose on pyruvate during compound F infusion. *J. Clin. Endocrinology and Metabolism*. **14**: 791-792.
  28. THORN, G. W., D. JENKINS, B. H. MCCracken, J. A. GARCIA-REYES & W. J. REDDY. 1952. Recent studies on ACTH and cortisone. *Trans. Assoc. Am. Phys.* **65**: 281-291.
  29. RECAN, L., D. H. HUME, P. H. FORSHAM & G. W. THORN. 1950. Effect of epinephrine on the pituitary adrenocortical system. *J. Clin. Endocrinol.* **10**: 187-229.
  30. SKELTON, F. R. 1950. On certain factors conditioning the action of the pituitary-adrenal system. *In Pituitary-Adrenal Function*: 141-42. Publ. Am. Assoc. Adv. Sci. Washington, D. C.
  31. JAILER, J. W., D. T. MARKS & P. A. MARKS. 1948. Variations in white blood cells following the oral administration of glucose to diabetics and nondiabetics. *J. Clin. Endocrinol.* **8**: 1074-1080.
  32. SCHNEEBERG, N. G. 1953. Does glucose stimulate adrenocortical activity? *Diabetes*. **2**: 372-375.
  33. REDDY, W. J., D. JENKINS & G. W. THORN. 1952. Estimation of 17-hydroxycorticoids in urine. *Metabolism*. **1**: 511-527.
  34. ABBOTT, W. E., H. KRIEGER, L. I. BOBB, S. LEVEY & W. HOLDEN. 1953. Metabolic alterations in surgical patients. I. The effect of altering the electrolyte, carbohydrate and amino acid intake. *Ann. Surg.* **138**: 434-452.
  35. MILLER, M., W. R. DRUCKER, J. E. OWENS, J. W. CRAIG & H. WOODWARD. 1952. Metabolism of intravenous fructose and glucose in normal and diabetic subjects. *J. Clin. Invest.* **31**: 115-125.
  36. PRICE, W. H., C. F. CORI & S. P. COLOWICK. 1945. The effect of anterior pituitary extract and of insulin on the hexokinase reaction. *J. Biol. Chem.* **160**: 633-634.
  37. DRUCKER, W. R., M. MILLER, J. CRAIG, W. MCK. JEFFERIES, S. LEVEY & W. E. ABBOTT.



1952. A comparison of the effect of operation on glucose and fructose metabolism. *Surgical Forum. Bull. Am. Coll. Surgeons.* : 548-555.
38. COREY, E. L. & S. W. BRITTON. 1939. Hypophyseal and adrenal interrelationships and carbohydrate metabolism. *Am. J. Physiol.* **126**: 148-154.
39. LONG, C. N. H. 1939. Diabetes mellitus in light of our present knowledge of metabolism. *Trans. & Studies Coll. Physicians Phila.* **7**: 21-46.
40. GRATTAN, J. F. & H. JENSEN. 1940. The effect of pituitary adrenocorticotrophic hormone and of various adrenal cortical principles on insulin hypoglycemia and liver glycogen. *J. Biol. Chem.* **135**: 511-517.
41. INGLE, D. J., H. A. WINTER, C. H. LI & H. M. EVANS. 1945. Production of glycosuria in normal rats by means of adrenocorticotrophic hormone. *Science.* **101**: 671-672.
42. HOUSSAY, E. D. & A. BIOSOTTI. 1936. Carbohydrate metabolism. *New Engl. J. Med.* **214** : 971-986.
43. EVANS, H. M., K. MYERS, M. E. SIMPSON & F. L. REICHERT. 1932. Disturbance of carbohydrate metabolism in normal dogs injected with hypophyseal growth hormone. *Proc. Soc. Exptl. Biol. Med.* **29**: 857-858.
44. BAUMANN, E. J. & D. MARINE. 1932. Glycosuria in rabbits following injections of saline extract of anterior pituitary. *Proc. Soc. Exptl. Biol. Med.* **29**: 1220-1223.
45. YOUNG, F. G. 1937. Permanent experimental diabetes produced by pituitary (anterior lobe) injections. *Lancet.* **2**: 372-374.
46. MCQUARRIE, I., E. G. BAUER, M. R. ZIEGLER & W. S. WRIGHT. 1949. Effects of pituitary adrenocorticotrophic hormone (ACTH) in children with non-Addisonian hypoglycemia. *Proc. Soc. Exptl. Biol. Med.* **71**: 555-559.
47. ABRAMS, G. D., B. L. BAKER, D. J. INGLE & C. H. LI. 1953. The influence of somatotropin and corticotropin on the islets of Langerhans of the rat. *Endocrinology.* **53**: 252-260.
48. COTES, P. M., E. REID & F. G. YOUNG. 1949. The diabetogenic action of pure anterior pituitary growth hormone. *Nature.* **164**: 209-211.
49. ABELOVE, W. A. & K. E. PASCHKIS. 1954. Comparison of the diabetogenic action of cortisone and growth hormone in different species. *Endocrinology.* **55**: 637-654.
50. CONN, J. W. 1947. The diagnosis and management of spontaneous hypoglycemia. *J. Am. Med. Assoc.* **134**: 130-137.
51. BROWN, G. M. 1951. The effect of ACTH in a case of beta islet-cell adenoma of the pancreas. *Am. J. Digest. Diseases.* **18**: 145-146.
52. SMITH, A. N. & J. B. COCHRAN. 1952. Islet-cell tumour of pancreas. Report of a case. *Lancet.* **1**: 289-293.
53. BROWN, H., H. P. HARGREAVES & F. H. TYLER. 1952. Islet-cell adenoma of the pancreas. *Arch. Internal Med.* **89**: 951-960.
54. MASON, A. S. 1953. Functioning malignant islet-cell tumour of pancreas. Primary growth removed, metastases in liver seen. Recurrence of hyperinsulinism, treated with cortisone. *Proc. Roy. Soc. Med.* **46**: 305-306.
55. OSNES, M. & R. G. THORSEN. 1953. Cortisone and corticotropin treatment in insuloma. *Acta Med. Scand.* **145**: 44-51.
56. ROXBURGH, R. C. 1954. Islet-cell adenoma of the pancreas in a child aged seven years. *Lancet.* **1**: 1057-1058.
57. DE PEYSTER, F. A. & R. K. GILCHRIST. 1954. Clinical response of spontaneous hypoglycemia to dietary and drug therapy. *J. Am. Med. Assoc.* **155**: 884-889.
58. BLACK, K. O., I. MACDONALD, E. REID & F. G. YOUNG. 1952. Trial of pituitary growth hormone in a case of hyperinsulinism due to islet-cell adenoma. *Lancet.* **1**: 19-21.
59. MCQUARRIE, I., M. R. ZIEGLER, W. S. WRIGHT, E. G. BAUER & R. A. ULSTROM. 1951. Further studies on the effects of ACTH on spontaneous hypoglycemia. *Proc. 2nd Clin. ACTH Conf.* : 69-80. J. R. Mote, Ed. Blakiston, Philadelphia, Pa.
60. GRIFFITHS, J. A. 1953. The effects of general anaesthesia, and hexamethonium, on the blood sugar in non-diabetic and diabetic surgical patients. *Quart. J. Med.* **22**: 405-418.
61. THORN, G. W., G. F. KOEPE, R. A. LEWIS & E. F. OLSEN. 1940. Carbohydrate metabolism in Addison's disease. *J. Clin. Invest.* **19**: 813-832.
62. THORN, G. W., D. JENKINS, J. LAIDLAW, F. GOETZ, J. DINGMAN, W. ARONS, D. H. P. STREETEN & B. H. MCCracken. 1953. Medical Progress: Pharmacologic aspects of adrenocortical steroids and ACTH in man. *New Engl. J. Med.* **248**: 232-245, 284-294, 323-337, 369-378, 414-423, 588-601, and 632-646.
63. CONN, J. W. 1952. Discussion of R. G. Sprague. Clinical use of adrenal cortical hormones and ACTH. *In Trans. 3rd Conf. on the Adrenal Cortex.* : 187. E. P. Ralli Ed. Josiah Macy, Jr. Found., New York, N. Y.

64. BAIRD, I. M. & D. S. MUNRO. 1954. Addison's disease with diabetes mellitus. A case treated with cortisone. *Lancet*. **1**: 962-964.
65. THORN, G. W. & P. H. FORSHAM. 1949. Metabolic changes in man following adrenal and pituitary hormone administration. *Recent Progr. Hormone Research*. **4**: 229-288.
66. SPRAGUE, R. G., C. F. GASTINEAU, H. L. MASON & M. H. POWER. 1948. Effects of synthetic 11-dehydrocorticosterone (compound A) in subject with Addison's disease. *Am. J. Med.* **4**: 175-185.
67. THORN, G. W., P. H. FORSHAM, T. F. FRAWLEY, D. L. WILSON, A. RENOLD, D. FREDRICKSON & D. JENKINS. 1951. Advances in the diagnosis and treatment of adrenal insufficiency. *Am. J. Med.* **10**: 595-611.
68. INGLE, D. J., J. E. NEZAMIS & E. H. MORLEY. 1951. Effect of continuous intravenous infusions of glucose upon work performance of adrenalectomized rats as related to fluid volume. *Am. J. Physiol.* **165**: 473-475.
69. THORN, G. W. 1949. Discussion of R. G. Sprague *et al.* Studies of the effects of adrenal cortical hormones on carbohydrate metabolism in human subjects. *Proc. Am. Diabetes Assoc.* **9**: 147-169.
70. BATES, M. W. & J. MAYER. 1952. Extension of glucostatic scheme of regulation of food intake to Houssay animals, diabetes and other special cases. *Federation Proc.* **11**: 436.
71. DURAS, F. P. 1951. Islet-cell tumour of the pancreas. *Brit. Med. J.* **1**: 702-703.
72. WEBSTER, D. D. & A. N. BLADES. 1952. Islet-cell tumour of the pancreas. *Brit. Med. J.* **1**: 307-308.
73. CONN, J. W., L. H. LOUIS & S. S. FAJANS. 1951. The probability that compound F (17-hydroxycorticosterone) is the hormone produced by the normal human adrenal cortex. *Science*. **113**: 713-714.
74. BOLAND, E. W. & N. E. HEADLEY. 1952. Compound F used orally in patients with rheumatoid arthritis. *J. Am. Med. Assoc.* **148**: 981-987.
75. INGLE, D. J. & R. C. MEEKS. 1952. Comparison of some metabolic and morphologic effects of cortisone and hydrocortisone given by continuous injection to rats. *Am. J. Physiol.* **170**: 77-80.
76. RUKES, J. M., R. H. ORR & P. H. FORSHAM. 1954. Clinical uses of intravenous hydrocortisone. *Metabolism, Clin. and Exptl.* **3**: 481-488.
77. INGLE, D. J. & J. E. NEZAMIS. 1949. Work performance of adrenally insufficient rats given adrenal cortex extract by continuous intravenous injection. *Am. J. Physiol.* **166**: 365-367.
78. INGLE, D. J., R. SHEPPARD, J. S. EVANS & M. H. KUIZENGA. 1945. A comparison of adrenal steroid diabetes and pancreatic diabetes in the rat. *Edocrinology*. **37**: 341-356.
79. HAUSBERGER, F. X. & A. J. RAMSAY. 1953. Steroid diabetes in guinea pigs. *Endocrinology*. **53**: 423-435.
80. SPRAGUE, R. G., H. L. MASON & M. H. POWER. 1949. Studies of the effects of adrenal cortical hormones on carbohydrate metabolism in human subjects. *Proc. Am. Diabetes Assoc.* **9**: 147-169.
81. BOOKMAN, J., S. R. DRACHMAN, L. E. SCHAEFER & D. ALDERSBERG. 1953. Steroid diabetes in man; the development of diabetes during treatment with cortisone and corticotropin. *Diabetes*. **2**: 100-111.
82. BISHOP, P. M. F. & J. H. GLYN. 1952. Diabetes caused by ACTH treatment of rheumatoid arthritis. *Proc. Roy. Soc. Med.* **45**: 168-170.
83. HIMWICH, W. A. & H. E. HIMWICH. 1946. Pyruvic acid in exercising depancreatized dogs and diabetic patients. *J. Biol. Chem.* **165**: 513-519.
84. HORWITH, M. K., O. W. HILLS & O. KREISLER. 1949. Lactic and pyruvic acids in the blood after glucose and exercise in diabetes mellitus. *Am. J. Physiol.* **166**: 92-99.
85. HILLS, O. W., M. H. POWER & R. M. WILDER. 1952. Diabetes mellitus and Cushing's syndrome. *Diabetes*. **1**: 351-357.
86. MCQUARRIE, I., W. H. THOMPSON & J. A. ANDERSON. 1936. Effects of excessive ingestion of sodium and potassium salts on carbohydrate metabolism and blood pressure in diabetic children. *J. Nutrition*. **11**: 77-101.
87. KINSELL, L. W., G. D. MICHAELS, S. MARGEN, L. BOLING & J. W. PARTRIDGE. 1952. Acceleration of neoglucogenesis from fat in response to ACTH and cortisone in human subjects. *Proc. West. Soc. Clin. Research*. *Am. J. Med.* **13**: 96-97.
88. KINSELL, L. W., H. E. BALCH & G. D. MICHAELS. 1953. Modification of "steroid diabetes" by potassium. *Metabolism, Clin. and Exptl.* **2**: 421-423.
89. WILSON, D. L. 1955. Personal communication.
90. KNIGHT, R. P., JR., D. KORNFELD, G. GLASER & P. K. BONDY. 1955. Effects of intra-

- venous hydrocortisone on electrolytes of serum and urine in man. *J. Clin. Endocrinol. and Metabolism*. **15**: 176-181.
91. FORSHAM, P. H. & G. W. THORN. 1949. Changes in inorganic serum phosphorus during the intravenous glucose tolerance test as an adjunct to the diagnosis of early diabetes mellitus. *Proc. Am. Diabetes Assoc.* **9**: 99-122.
  92. HAYES, M. A. & R. L. BRANDT. 1952. Carbohydrate metabolism in the immediate postoperative period. *Surgery*. **32**: 819.
  93. KUPPERMAN, H. S., M. PERSKY, J. LINSKY, M. ISAACS & M. ROSENBLUTH. 1955. The paradoxical effect of intravenous hydrocortisone upon carbohydrate metabolism. *Ann. N. Y. Acad. Sci.* **61**(2): 494-501.
  94. BASTENIE, P. A., P. SPEHL, V. CONRAD, M. VERBIST & J. R. M. FRANCKSON. 1953. Anti-diabetic effect of cortisone in certain cases of steroid diabetes. *Acta Med. Scand.* **145**: 341-356.

# THE PARADOXICAL EFFECT OF INTRAVENOUS HYDROCORTISONE UPON CARBOHYDRATE METABOLISM

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The effect of intravenous hydrocortisone upon carbohydrate tolerance was studied in man in an attempt to characterize further the activity of this steroid in clinical application. These studies were predicated, in part, upon previous clinical investigations on the effect of intravenous infusion of ACTH upon glucose tolerance and insulin sensitivity.<sup>1, 2</sup> We were able to demonstrate decreased glucose tolerance and insulin resistance with different dose levels of several ACTH preparations. The linear response, however, with respect to changes in glucose tolerance and insulin sensitivity at the various doses employed was neither precise nor exact.<sup>1, 2</sup> It was finally established that assay of ACTH in the human could not be accurately quantitated by noting the degree of changes or responsiveness in carbohydrate tolerance after ACTH infusion.<sup>2</sup>

Comparable studies with intravenous hydrocortisone were undertaken. The effect of the adrenal steroid upon carbohydrate tolerance was studied and was based upon its anticipated effect on glucose and insulin tolerance as described by other investigators in animals and in humans.<sup>3-9</sup> It has been generally accepted that cortisone or cortisonelike derivatives will induce decreased glucose tolerance and insulin resistance. We are interested in exploiting these properties of hydrocortisone to see whether a linear dose response could be established between the degree of induced decreased glucose tolerance or insulin resistance and the dose of hydrocortisone administered. During the process of this experimental study, several facts were soon established. First, it was difficult to quantitate a dose-response relationship between the administered hydrocortisone and blood-glucose levels when glucose tolerance was studied. Second, we were surprised to note that, despite the consistent decrease in glucose tolerance achieved by the intravenous hydrocortisone, there was no evidence that intravenously administered hydrocortisone could alter insulin responsiveness. It is this paradoxical effect of hydrocortisone on carbohydrate tolerance which will be discussed in this report.

Hospitalized patients with either normal or decreased glucose tolerance were subjects of this study. The patients with decreased glucose tolerance were selected from a group of rheumatoid arthritics not showing any manifestations of metabolic disturbances. Both groups of patients were subjected to the following procedures.

(1) *Glucose tolerance test.* The patients received a standard single oral glucose load (100 grams), following which blood for glucose and phosphorus determinations was taken at 30 to 60 minute intervals for 3 hours.



(2) *Hydrocortisone (free alcohol) tolerance test.* One hundred mgm. of hydrocortisone dissolved in 20 cc. of 50 per cent alcohol were added to a 500 cc. flask of normal saline solution. The infusion was started and permitted to run for four hours at a constant and continuous rate. Blood was taken at 30 and 60 minute intervals for determination of blood glucose and phosphorus.

(3) *Hydrocortisone-glucose tolerance test.* Procedures 1 and 2 were combined and performed simultaneously in the following manner. The patients received 100 grams of glucose orally and were immediately started on the hydrocortisone infusion, which was permitted to run for four hours. Blood was taken at the prescribed intervals and the same determinations as above were obtained. The true glucose values were derived on all blood samples, while the method of Youngberg and Youngberg<sup>10</sup> was employed for the blood phosphorus studies.

(4) *Insulin-hydrocortisone tolerance test.* During a four-hour infusion with hydrocortisone, a series of patients received an intravenous injection of insulin, 0.1 unit of regular insulin/kg. of body weight, after the hydrocortisone infusion had been in effect for a period of two hours. The insulin was administered at the two-hour mark, since a significant decrease in glucose tolerance had been demonstrated at this time. It was also noted that, when the blood eosinophile response was studied by quantitative counts, a significant drop in blood eosinophiles was achieved after two hours of hydrocortisone infusion had been completed.

One should note that all patients were maintained on high carbohydrate diet prior to and during the experimental procedure. At least a five- to six-day rest period was instituted between each test. Changes in blood glucose were recorded as percentage of deviation from the control or preinfusion level where control values are considered as the 100 per cent level.

### Results

While the hydrocortisone infusion alone induced insignificant changes in blood glucose, the simultaneous oral administration of glucose, at the beginning of the hydrocortisone infusion, resulted in a significant decrease in glucose tolerance in patients with either decreased or normal glucose tolerance (FIGURE 1 and TABLE 1). Of the eight patients with normal glucose tolerance, only one showed a lower blood-glucose level at the third hour during the combined glucose tolerance and intravenous infusion with hydrocortisone as compared to the three-hour reading obtained during performance of glucose tolerance alone. The blood glucose of this patient, however, increased so that, at the end of four hours of infusion with hydrocortisone, the blood sugar value was higher than that noted after the three hours' reading during the glucose-tolerance test alone. The extent of decrease in glucose tolerance induced by the hydrocortisone in the patients, initially, with decreased glucose tolerance was comparable to that noted in the patients with normal tolerance (FIGURE 1).

Blood eosinophile counts were determined during the hydrocortisone infusion utilizing the method of Thorn *et al.*<sup>11</sup> A significant fall in blood eosinophiles was achieved at the end of two hours of infusion. The blood eosinophile count

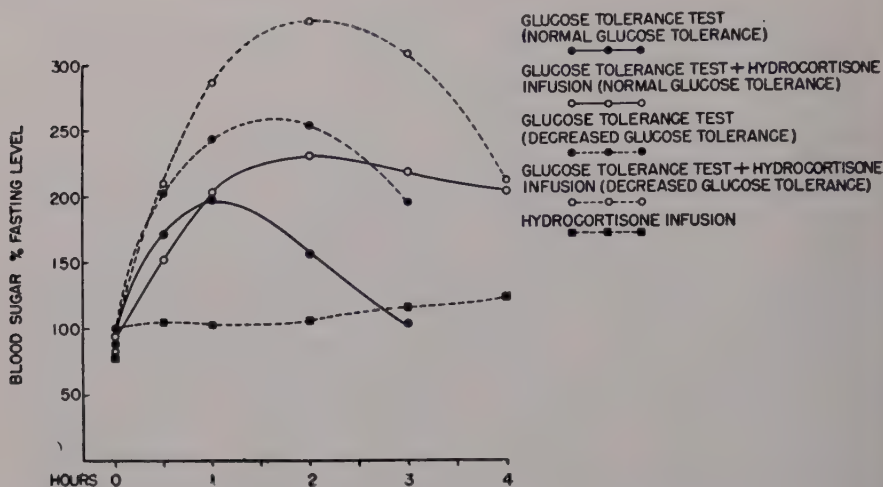


FIGURE 1. Effect of hydrocortisone infusion in patients with normal and decreased glucose tolerance.

averaged near the zero level at the end of the four-hour infusion period (FIGURE 2).

The effect of the hydrocortisone infusion upon insulin responsiveness is depicted in FIGURE 3 and TABLE 1. Here it is shown that adequate insulin responsiveness was obtained, since the blood-glucose value fell more than 50 per cent of the preinsulin level within 30 minutes after insulin administration. Insulin was administered presumably during effective activity of the hydrocortisone infusion, as evidenced by the fact that significant decrease in glucose tolerance and fall in blood eosinophiles had already been achieved at the time the insulin was injected (FIGURES 1 and 2). The two-hour period of infusion was associated with a significant fall in blood eosinophiles and notable decrease in glucose tolerance. The insulin responsiveness that was observed cannot be ascribed to the administration of insulin at a time when there was an absence of adequate hydrocortisone activity. The insulin responsiveness was identical in control patients and in those receiving a simultaneous infusion with hydrocortisone (FIGURE 3).

In an attempt to characterize further the action of hydrocortisone upon carbohydrate tolerance, blood phosphorus studies were obtained. Blood phosphorus levels in patients subjected to the above experimental procedure revealed that, in all groups, there was a definite decrease in blood phosphorus (FIGURE 4) during the hydrocortisone infusion, regardless of the administration of glucose and/or insulin. An accentuated drop in blood phosphorus was observed in the patients receiving the combined glucose-hydrocortisone test (FIGURE 4). The decrease in blood phosphorus may be considered as an indication of peripheral utilization of glucose<sup>12, 13</sup> and would imply that the changes induced in carbohydrate tolerance cannot be attributed to decreased peripheral utilization of glucose.<sup>14</sup> Administration of insulin also induced a decrease in blood phosphorus, despite the preceding and concomitant infusion of hydro-

TABLE 1

THE EFFECT OF INFUSION OF HYDROCORTISONE UPON GLUCOSE TOLERANCE AND INSULIN SENSITIVITY IN PATIENTS WITH NORMAL GLUCOSE TOLERANCE

Procedure	Time after glucose administration or initiation of infusion								
	0	30 Min.	60 Min.	120 Min.	150 Min.	180 Min.	210 Min.	240 Min.	
Glucose tolerance	100 (8)	173 ± 10.2 (8)	197 ± 10.7 (8)	157 ± 160 (8)	—	104.5 ± 10.9 (8)	—	—	
Hydrocortisone infusion	100 (9)	105.5 ± 3.6 (9)	104.5 ± 3.3 (9)	108 ± 6.6 (9)	—	116.6 ± 5.2 (9)	—	124 ± 12.1 (6)	
Glucose tolerance + hydrocortisone infusion	100 (8)	152.8 ± 16.4 (8)	211.6 ± 17.2 (8)	229 ± 19.8 (8)	—	223 ± 36.5 (8)	—	204.6 ± 33.6 (8)	
Insulin tolerance† + hydrocortisone infusion	100 (6)	—	102 ± 3.4 (6)	110 ± 5.8 (5)	47.5 ± 6.9 (6)	77.3 ± 5.6 (6)	112 ± 5.3 (4)	—	
Insulin tolerance‡	—	—	—	100	50	78	93	—	

Figures in parentheses refer to number of patients.

\* Per cent of fasting glucose  $\pm$  S. E.

† 0.1 Unit regular insulin/kg. of body weight intravenously at 120 minutes.

‡ Average readings from large series of control patients.

cortisone (FIGURE 3). Comparable changes in blood phosphorus and peripheral utilization of glucose have been reported by others after administration of cortisone or adrenal cortical extracts.<sup>15, 16</sup>

The paradoxical effect and discrepancy noted between glucose tolerance and insulin responsiveness after hydrocortisone infusion is difficult to ascribe to known mechanisms of action of hydrocortisone upon carbohydrate function. The decreased glucose tolerance that was observed may be attributed in part to the catabolic effect of the 11-oxygenated corticoids resulting in gluconeogenesis from protein catabolism.<sup>17</sup> The acute nature of these studies, however, raises a question of doubt as to the acceptability of the catabolic action of hydrocortisone as the *modus operandi* in inducing decreased glucose tolerance in our patients.

The possible effect of hydrocortisone upon inhibition of the hexokinase system must be considered as a possible factor in decreasing glucose tolerance.<sup>18</sup> The same mechanism however, would be expected also to induce insulin re-

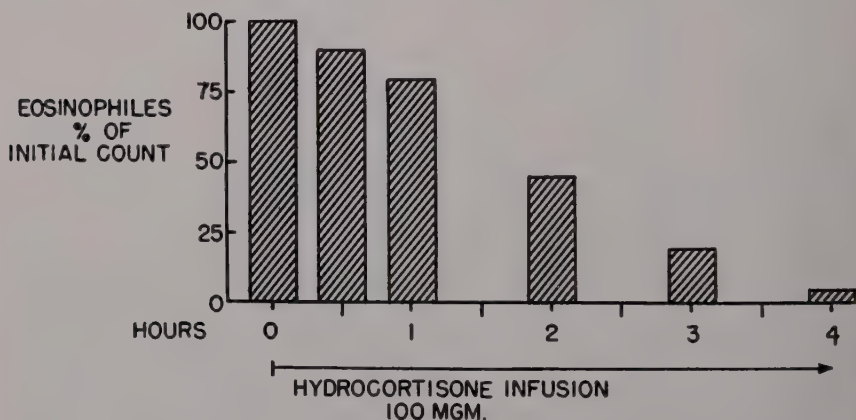


FIGURE 2. Effect of hydrocortisone infusion upon eosinophile count in man.

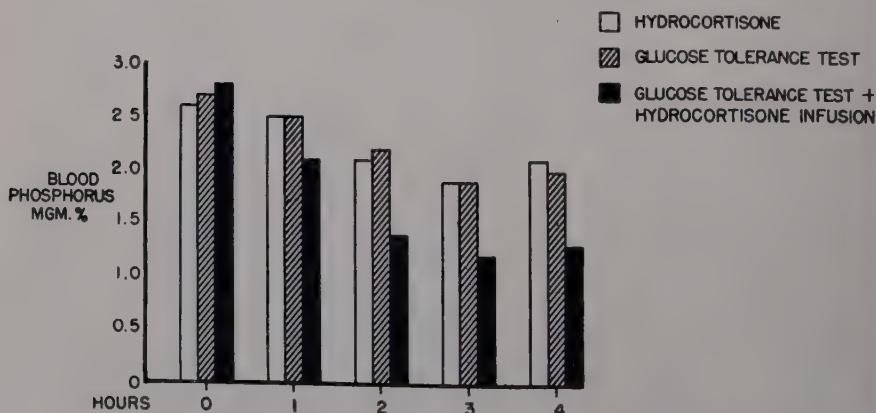


FIGURE 3. Effect of glucose tolerance and hydrocortisone infusion upon blood phosphorus.



sistance. This difference, in effect, may be explained, in part, on the basis that, while the hydrocortisone may effect the enzyme system necessary for the activity of endogenous insulin, the adrenal cortical steroid is not capable of inhibiting the action of exogenous insulin. It is conceivable that the administered insulin (exogenous) is not identical with endogenous insulin secreted by the pancreas and, while apparently comparable physiological effects are achieved by these insulins, their reaction upon enzyme systems may differ.

One must consider also the possibility that induction of decreased glucose tolerance without influencing insulin sensitivity can be attributed partly to the fact that the hydrocortisone infusion may cause a release of an anti-insulin factor, *i.e.*, the hyperglycemic factor from the pancreas.<sup>19-21</sup> This hormone from the alpha cells would accentuate the alimentary hyperglycemia and provide thereby a decrease in glucose tolerance. The hyperglycemic factor, however, may well be unable to inhibit the response of the organism to exogenous insulin in the absence of a glucose load.

One last possibility that must be considered is that hydrocortisone may have an effect upon the release of insulin from the pancreatic islet cells. Thus, under the stimulus of alimentary hyperglycemia, one would ordinarily anticipate a release of insulin from the pancreas. This might well be prevented by hydrocortisone and would result, thereby, in decreased glucose tolerance under a standard glucose load. Since only release of insulin is prevented according to this hypothesis, however, responsiveness to exogenous insulin is not inhibited. Hence, while hydrocortisone may inhibit the release of endogenous insulin, the

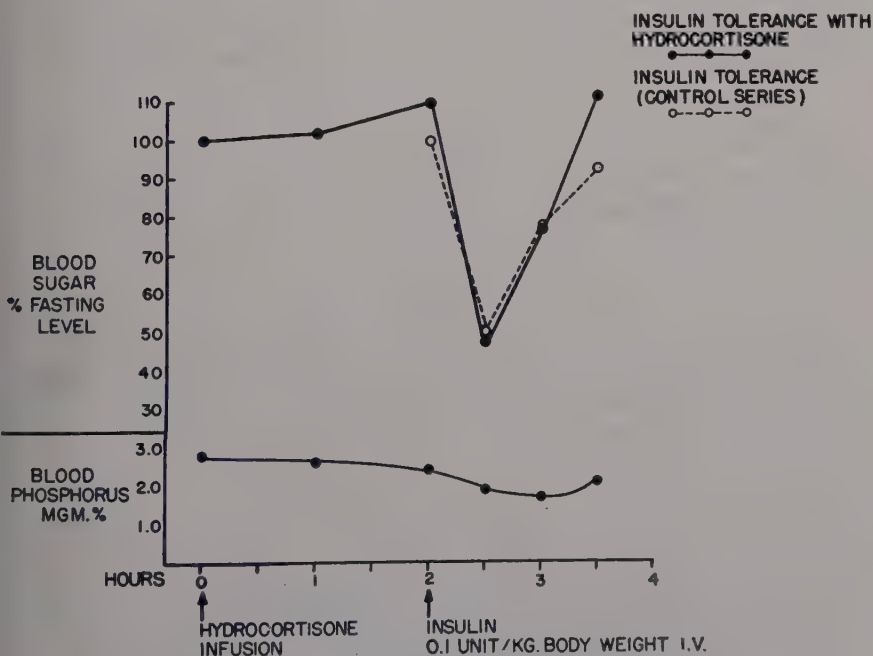


FIGURE 4. Effect of hydrocortisone infusion upon response to insulin.

administration of exogenous insulin under the same conditions can result in a normal insulin responsiveness without evidence of anti-insulin activity, since endogenous pancreatic insulin secretion is not necessary in the blood glucose response of the organism to exogenous insulin. The final explanation will have to await further studies.

### *Summary*

The effect of intravenous infusion of 100 mgm. of hydrocortisone upon carbohydrate tolerance was studied in a series of hospitalized patients with normal or decreased glucose tolerance. The infusion was administered over a period of four hours, blood being obtained at prescribed intervals for glucose and phosphorus determinations. Hydrocortisone infusion itself had no appreciable effect upon blood-glucose levels. Oral administration of glucose plus the intravenous infusion of hydrocortisone, however, resulted in a marked decrease in glucose tolerance in patients who had previously demonstrated normal or decreased glucose tolerance. The blood-phosphorus levels in all patients showed a fall, indicating peripheral utilization of glucose. Sensitivity to insulin during the hydrocortisone infusion was studied by intravenous administration of 0.1 unit of regular insulin per kilogram of body weight after two hours of infusion with hydrocortisone. Blood was then obtained at half-hour intervals thereafter for another two hours, during which time the hydrocortisone infusion was continued. It was noted that, despite the fact that hydrocortisone decreased the tolerance of patients to oral administration of glucose, the infusion did not inhibit the hypoglycemic effect of the intravenously administered insulin. The insulin tolerance response was identical in patients with or without hydrocortisone. The implications of these findings are discussed.

### *Acknowledgments*

Appreciation is expressed to Gwendolyn Newhook, research nurse, for valuable assistance rendered during this investigation. The hydrocortisone used in this study was kindly supplied by Doctor H. F. Hailman of the Department of Clinical Investigation, the Upjohn Company, Kalamazoo, Mich.

### *References*

1. APPEL, S. B., J. L. GLUCK, S. REICHMAN, A. MILLER, A. GOLDMAN, C. SPRINGER, A. A. SCHLECKER, M. B. ROSENBLUTH & H. S. KUPPERMAN. 1952. Effect of adrenocorticotrophic hormone on blood glucose and insulin hypoglycemia. *Federation Proc.* **11**: 1.
2. APPEL, S. B., J. L. GLUCK, A. A. SCHLECKER, A. MILLER, S. REICHMAN, C. SPRINGER, A. GOLDMAN, M. B. ROSENBLUTH & H. S. KUPPERMAN. 1953. The comparative effect of adrenocorticotrophic hormone extracts on blood glucose in man. *Acta Endocrinol.* **14**: 99.
3. SOSKIN, S. & R. LEVINE. 1952. *Carbohydrate Metabolism*. Univ. Chicago Press, Chicago, Ill.
4. GRATTAN, J. F. & H. JENSON. 1940. The effect of the pituitary adrenocorticotrophic hormone and various adrenal cortical principles on insulin hypoglycemia and liver glycogen. *J. Biol. Chem.* **135**: 511.
5. INGLE, D. J. 1941. The production of glycosuria in the normal rat by means of 17-hydroxy-11-dehydrocorticosterone. *Endocrinology*. **29**: 649.
6. CONN, J. W., H. L. LAWRENCE & M. W. JOHNSTON. 1948. Studies upon mechanisms involved in the induction with adrenocorticotrophic hormone of temporary diabetes mellitus in man. *Proc. Am. Diabetes Assoc.* **8**: 213.

7. SPRAGUE, R. G., M. H. POWER, H. L. MASON, A. ALBERT, D. R. MATHIESON, P. S. HENCH, E. C. KENDALL, C. H. SLOCUMB & H. F. POLLEY. 1950. Observations on the physiological effects of cortisone and ACTH in man. *Arch. Internal Med.* **85**: 199.
8. CONN, J. W., S. F. STEPHAN, H. L. LAWRENCE & B. JOHNSON. 1951. Metabolic and clinical effects of corticosterone (compound B) in man. *Proc. 2nd ACTH Clin. Conf.* **1**: 221.
9. BUNIN, J. J., A. J. KALTMAN & C. McEWEN. 1952. Diabetogenic effect of cortisone and ACTH in a non diabetic patient with rheumatoid arthritis. *Am. J. Med.* **12**: 125.
10. YOUNGBERG, G. E. & M. V. YOUNGBERG. 1930. Phosphorus metabolism; system of blood phosphorus analysis. *J. Lab. Clin. Med.* **16**: 158.
11. THORN, G. W., P. H. FORSHAM, F. T. G. PRUNTY & A. G. HILLS. 1948. A test for adrenal cortical insufficiency. *J. Am. Med. Assoc.* **137**: 1005.
12. FORSHAM, P. H. & G. W. THORN. 1949. Changes in inorganic serum phosphorus during the intravenous glucose tolerance test as an adjunct to the diagnosis of early diabetes mellitus. *Proc. Am. Diabetes Assoc.* **9**.
13. INGBAR, S. H., E. H. KASS, C. H. BURNETT, A. S. RELMA, B. A. BURROUGHS & J. H. SISSON. 1951. The effects of ACTH and cortisone on the renal tubular transport of uric acid, phosphorus and electrolytes in patients with normal renal and adrenal function. *J. Lab. Clin. Med.* **38**: 533.
14. CONN, J. W. 1953. Endocrine regulation of blood sugar. *Ann. Internal Med.* **38**: 179.
15. MACBRYDE, C. M. & F. A. DE LA BALZE. 1944. Pork adrenal cortical extract. Effect upon carbohydrate metabolism and work capacity in Addison's disease. *J. Clin. Endocrinol.* **4**: 287.
16. INGLE, D. J., M. C. PRESTRUD, J. E. NAZAMIS & M. H. KUIZENGA. 1927. Effect of adrenal cortex extract upon the tolerance of the eviscerated rat for intravenously administered glucose. *Am. J. Physiol.* **150**: 423.
17. LONG, C. N. H. 1939. Diabetes mellitus in the light of our present knowledge of metabolism. *Trans. & Studies Coll. Physicians Phila.* **7**: 21.
18. COLOWICK, S. P., G. T. CORI & M. W. STEIN. 1947. The effect of adrenal cortex and anterior pituitary extracts and insulin on the hexokinase reaction. *J. Biol. Chem.* **168**: 583.
19. FOA, P. P., H. R. WEINSTEIN & J. A. SMITH. 1949. Secretion of insulin and of a hyperglycemic substance studied by means of pancreatic femoral cross circulation experiments. *Am. J. Physiol.* **157**: 197.
20. SUTHERLAND, E. W. 1950. The effect of the hyperglycemic factor of the pancreas and of epinephrine on glycogenolysis. *Recent Progress in Hormone Research.* **5**: 441.
21. FOA, P. P. 1954. Glucogen, the hyperglycemic glycogenolytic hormone of the pancreas. *Advances Internal Med.* **6**: 29-58.

## B. JOINT INJECTION

### STUDIES ON THE METABOLISM OF ADRENAL CORTICAL STEROIDS IN THE SYNOVIAL CAVITY IN RHEUMATOID ARTHRITIS\*

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The effectiveness of hydrocortisone in suppressing inflammation in rheumatoid joints when injected intra-articularly is well established. Since the synovial fluid of inflamed knee joints can be readily withdrawn by aspiration, an opportunity is presented for direct observation of the chemical alterations produced in the hormone by the tissue on which it is acting. Moreover, this technique makes it possible to follow the metabolism of other steroid substances *in vivo* by a peripheral tissue, in both normal and diseased states. Such studies could indicate whether the anti-inflammatory effect of hydrocortisone involves chemical transformation of the steroid or is independent of it.

It is well known that, when administered systemically, cortisone and hydrocortisone do not differ greatly in their ability to suppress the inflammatory reactions of rheumatoid arthritis. Upon being injected directly into the synovial cavity, however, cortisone has little or no effect, while hydrocortisone exhibits local anti-inflammatory power. This difference is in contrast to the local effectiveness of both steroids in some other inflammatory conditions. The ineffectiveness of cortisone in the synovial cavity suggests that chemical transformations are involved in anti-inflammatory activity, and that these cannot be adequately effected by synovial tissue when cortisone is injected. It was hoped, therefore, that a comparative study of the metabolites produced from cortisone and hydrocortisone might throw light on the mechanisms by which their anti-inflammatory activities are exerted.

#### *Materials and Methods*

*Subjects.* All subjects had rheumatoid arthritis with effusions in one or both knees. All, incidentally, were receiving maintenance therapy of 75 or 100 mg. cortisone acetate by mouth daily in four divided doses.

*Intra-articular injection and collection of synovial fluid.* Fifty or 100 mg. of cortisone, hydrocortisone, or their acetates, were injected intra-articularly in saline suspensions. After a definite time interval, all the fluid was aspirated and the joint cavity was washed several times with saline. The combined fluids and washings were then extracted with chloroform and analyzed for corticosteroids. At the same time, fluid from the uninjected contralateral knee was analyzed as a control.

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TABLE 1

DISAPPEARANCE OF CORTISONE AND HYDROCORTISONE AFTER INTRA-ARTICULAR INJECTION

Hours after intra-articular injection	Compound	Mg. injected	Per cent disappearance (average)
$\frac{1}{2}$	F	100	78
1	F <sub>Ac</sub>	50	86
1	E <sub>Ac</sub>	50	
3	F	100	97
3	E	100	

F—hydrocortisone (free alcohol); E—cortisone (free alcohol); F<sub>Ac</sub>—hydrocortisone acetate; E<sub>Ac</sub>—cortisone acetate.

### Results

*Rate of disappearance of injected steroids.* We have previously noted the rapidity with which the injected steroids disappeared from the synovial fluid.<sup>1</sup> As judged by Porter-Silber assay,<sup>2</sup> cortisone, hydrocortisone, and their acetates were all removed from the synovial fluid or metabolized to nonreactive compounds at essentially the same rate.

TABLE 1 gives the results of five such analyses. It is seen that, half an hour after the intra-articular injection of 100 mg. of hydrocortisone (in the form of the free alcohol), 78 per cent had already disappeared. After one hour, 86 per cent of 50 mg. of hydrocortisone acetate could no longer be recovered, and the same was true for cortisone acetate. By the end of three hours, 97 per cent of both cortisone and hydrocortisone had disappeared. This rapid rate of disappearance has been demonstrated also by Zacco and his co-workers<sup>3</sup> using a similar technique, and by Gallagher *et al.*<sup>4</sup> using labelled cortisone. Subsequent assays after chromatography and elution revealed additional material which reacted in the chromic acid-ketosteroid assay for 17-hydroxy-corticosteroids and 17-ketosteroids.<sup>5</sup> These substances would increase the steroid remaining after three hours to a maximum of about 8 per cent.

*Appearance of metabolites in the synovial fluid.* It was of interest to determine whether the small amount of remaining corticosteroid contained metabolic products in addition to the unchanged injected steroid. Accordingly, the total neutral fractions of the chloroform extracts were chromatographed on paper by the technique of Zaffaroni, Burton, and Keutmann,<sup>6</sup> using the toluene-propylene glycol system. Chromatograms of postinjection fluids have repeatedly shown several bands in addition to the unchanged injected substance. It was necessary, of course, to be certain that these other substances were not present in the synovial fluid before injection. This also has been consistently found true, and extracts of amounts of control fluid up to 130 ml., chromatographed on very narrow papers, have yielded either no bands or very faint ones. Hence, the additional materials found after hormone injection must have originated from the injected steroids.

FIGURE 1 illustrates one of these experiments. In this figure, the pointed strips represent papers containing the compounds of slow, medium, and fast mobility in the toluene-propylene glycol system. The narrow strips represent 1 cm. papers, and the wider strips, 10 cm. papers. The bands indicate where

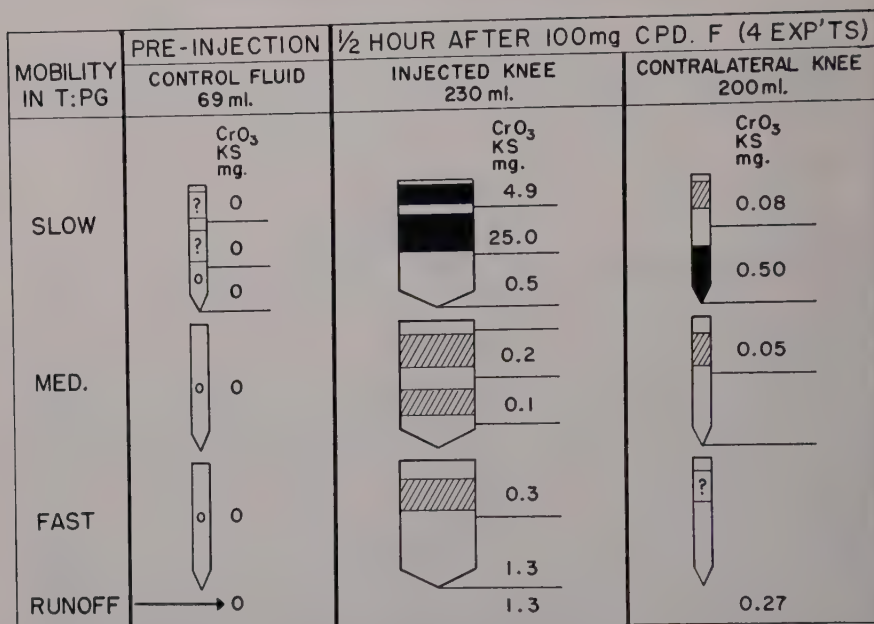


FIGURE 1

Chromatograms of extracts of synovial fluid aspirated one half-hour after the intra-articular injection of hydrocortisone (free alcohol). Total amount injected = 400 mg.

T:PG is toluene: propylene glycol, F is hydrocortisone free alcohol, CrO<sub>3</sub>-KS is titers by the chromic acid-ketosteroid determination.<sup>6</sup>

The black bands show where strong reducing tests were obtained on the paper with triphenyltetrazolium chloride. The shaded bands are weak tests.

The wide band assaying 25.0 mg. on the center chromatogram was identified as unchanged hydrocortisone.

"Slow" papers were developed for seven days, "medium" papers for two days, and "fast" papers for five hours.

spot tests were obtained with triphenyltetrazolium chloride, which gives a red color with corticosteroids possessing reducing side chains. The figures to the right of each paper show the titer of each band in milligrams by the chromic acid-ketosteroid assay.

The left-hand chromatogram of FIGURE 1 shows that the pre-injection control fluid yielded no definite reducing bands, nor were any steroids revealed by elution and assay. In contrast, one half-hour after the injection of 100 mg. of hydrocortisone free alcohol (center chromatogram), at least four reducing bands, in addition to the 25.0 mg. of unchanged hydrocortisone, were seen. In addition, at least three 17-ketosteroids were found on the fast paper and in the runoff.

*Evidence that these metabolites are formed in the synovial cavity.* It was important to ascertain whether these metabolites were formed locally in the knee, or elsewhere, as in the liver, from the hydrocortisone which had been absorbed from the knee.

If the latter were the case, one would expect to find the same compounds in similar amounts in the contralateral uninjected knee. The right-hand chromatograms of FIGURE 1, however, show that the metabolites were far less abundant in the contralateral knee fluid. In spite of being spread on a chro-

matogram 10 times as wide, the postinjection extract gave more and stronger bands, and the titer of metabolites totalled 8.6 mg., exclusive of the unchanged hydrocortisone. Only 0.9 mg. in all was found in the contralateral fluid and, of this, the strong band assaying 0.5 mg. is probably unchanged hydrocortisone, rather than a metabolite. Hence, it was concluded that the metabolites are chiefly formed locally, in the knee itself.

*Metabolism of hydrocortisone by synovial fluid and tissue in vitro.* Other evidence for the local formation of metabolites is provided by preliminary incubation experiments. Strips of freshly excised synovial tissue were incubated in saline solution for three hours with 10 mg. of added hydrocortisone suspension. After extraction and chromatography, several definite new bands giving typical steroid reactions were found. This was true with synovial tissue from both normal and rheumatoid subjects.

The enzymes responsible for these transformations also appeared to be present in rheumatoid synovial fluid, since incubation of 10 mg. hydrocortisone with 15 ml. whole fluid yielded well-localized new bands which were either much weaker or entirely absent when the same fluid was incubated alone. That the tissue itself, rather than the fluid, is responsible for the major portion of the metabolic reactions *in vivo*, is indicated by the relatively small amounts of metabolites produced by incubating whole fluid with hydrocortisone.

#### *Patterns of Metabolites in Synovial Fluid*

*Experiment A: One half-hour after the injection of hydrocortisone (free alcohol).*

The chromatograms of these extracts are shown in simplified form in FIGURE 1. It may be seen that a variety of metabolites appeared in the chloroform extract of synovial fluid. Their mobilities varied from that of the very slow band which scarcely moved at all, to 17-ketosteroid material moving faster than androsterone. Some of these compounds were revealed by the reduction spot test with triphenyltetrazolium chloride on the paper, others only by micro assay after elution.

Unchanged hydrocortisone was recovered to the extent of about 5 per cent, whereas the total titer of metabolites by the chromic acid-ketosteroid assay was about 2 per cent of the injected hydrocortisone. At least three corticosteroid metabolites, of both lesser and greater polarity than hydrocortisone, and four 17-ketosteroids were found. The corticosteroid metabolites were unknown compounds which could not be identified by the techniques at our disposal.

The very slow-moving metabolite assaying 4.9 mg. (FIGURE 1) is of particular interest, since its mobility cannot be accounted for except by the introduction of new polar groups or configurations. This would be in contrast to the usual degradative metabolic reactions. Various tests and assays gave evidence for the following structural features: an  $\alpha$ -ketol was indicated by the reduction test on the paper. A 17-hydroxy- $\alpha$ -ketol was shown by the formation of a 17-ketosteroid on oxidation of the free compound with chromic acid, but not after acetylation. A  $\Delta^4$ -3-ketone was shown by a characteristic absorption curve in ethanol with a good peak at 240  $\mu$ . The slowest known  $C_{21}O_5$  steroid having all these structural features is 11 $\alpha$ -hydrocortisone, whose sulfuric

acid chromogen absorption spectrum gives peaks at 237, 282, 391, and 475  $m\mu$ .<sup>7</sup> The metabolite gave peaks at 285, 325, 380, and 465  $m\mu$ , somewhat resembling the spectrum of 11 $\alpha$ -hydrocortisone except for the peak at 325  $m\mu$ . Rechromatography of the acetate suggested that two compounds might be present, only one of which absorbed at 325  $m\mu$ . An alternative explanation of the slow mobility of this material would be that an additional oxygen group had been introduced, resulting in a  $C_{21}O_6$  steroid, though the sulfuric acid chromogen spectrum is not that of 6 $\beta$ -hydroxy-hydrocortisone.<sup>8</sup>

The next reducing band, assaying 0.2 mg. was not investigated, while the faster reducing band on the medium paper, assaying 0.1 mg. was an unknown corticosteroid of the same mobility as cortisone. The reducing band at the top of the fast paper, assaying 0.3 mg., moved parallel to corticosterone, but was shown not to contain that compound, since chromic acid oxidation of its acetate did not yield 11-dehydro-corticosterone.

Elution of the lower portion of the fast paper yielded material assaying 1.3 mg. in the chromic acid-ketosteroid determination. This was identified as  $\Delta^4$ -androstene-11 $\beta$ -ol-3,17-dione, the 17-ketosteroid corresponding to hydrocortisone. Less polar 17-ketosteroid material was detected in the runoff by the assay procedure and, on rechromatography in ligroin-propylene glycol, three regions reacting to alkaline m-dinitrobenzene were seen.

The right-hand chromatogram of FIGURE 1 was obtained from an extract of the contralateral, uninjected knee, which was aspirated at the same time as the injected knee (one half-hour post injection). It is apparent that definitely more compounds were present than in extracts of preinjection control fluids (*cf.* left-hand chromatogram of FIGURE 1). It is therefore probable that the injected steroid and, possibly, its metabolites diffused through the circulating body fluids and entered the contralateral synovial cavity. The chief constituent of the contralateral fluid was shown most probably to be hydrocortisone, in amount roughly 0.1 per cent of that injected (the strong reducing band at the bottom of the slow paper, assaying 0.5 mg.).

*Experiment B: Three hours after the injection of hydrocortisone.* The chromatogram of this extract is shown in the left-hand section of FIGURE 2. There were fewer metabolites than after one half-hour and the most abundant products were different. The slow moving fraction seen after one half-hour was no longer present in appreciable concentration. Unchanged hydrocortisone had diminished to 0.4 mg. or 0.1 per cent of that injected.

The dominant metabolite was contained in a band of medium mobility travelling between cortisone and Reichstein's substance S. The total amount of this fraction exceeded that of the unchanged hydrocortisone. Cortisone was not detected in this band, or elsewhere. No faster moving material was detected by chromic acid-ketosteroid assay. Thus, the 17-ketosteroids which were present after one half-hour had either been absorbed or further broken down.

*Experiment C: Three hours after the injection of cortisone.* The chromatogram of this extract is shown in the right-hand section of FIGURE 2. The pattern of metabolites was quite different from that found after hydrocortisone.



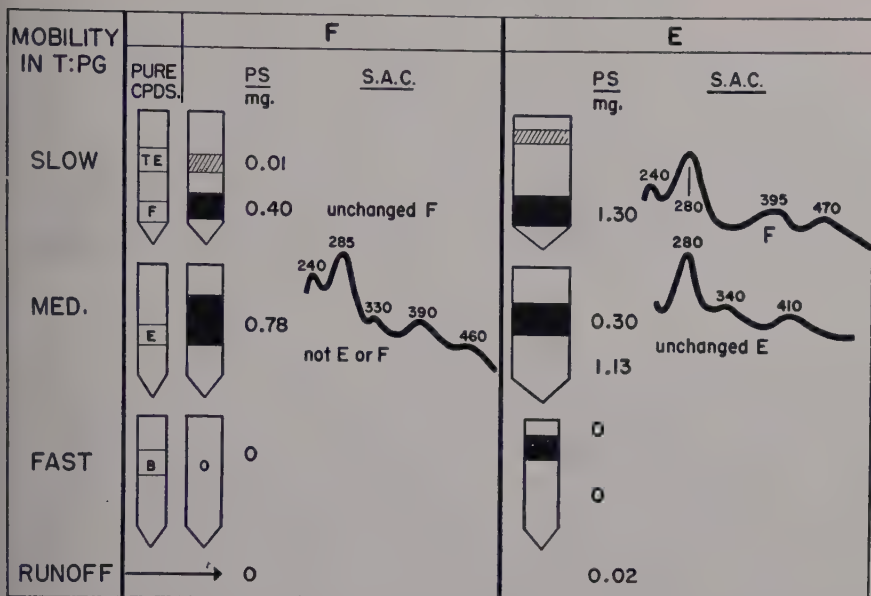


FIGURE 2

Comparison of metabolites after the intra-articular injection of hydrocortisone and cortisone (free alcohols), aspirated three hours after the injection of 100 mg. Four experiments combined. Total amount injected = 400 mg.

TE is tetrahydro cortisone, F is hydrocortisone, E is cortisone, B is corticosterone.

PS is titer by Porter-Silber assay; S.A.C. is sulphuric acid chromogen spectrum in  $m\mu$ .

For explanation of other terms see FIGURE 1.

The most polar band, at the top of the slow paper, was different from the material of similar mobility seen one half-hour after hydrocortisone. Its sulfuric acid chromogen gave good peaks at 285 and 400  $m\mu$ , indicating a  $\Delta^4$ -3-ketone, but was not that of the  $20\alpha$ -hydroxy steroid corresponding to cortisone. Possibly a  $C_{21}O_6$  steroid was present.

The most noteworthy metabolite of cortisone in this experiment was hydrocortisone, which was identified in the lower reducing band on the slow paper. The 1.3 mg. of new hydrocortisone found by Porter-Silber assay exceeded the 0.3 mg. of injected cortisone remaining unchanged.

The most plentiful conversion product of cortisone was a nonreducing steroid which ran just ahead of the cortisone band. This material reacted to some extent in both the Porter-Silber and chromic acid-ketosteroid determinations (1.13 and 2.4 mg. respectively) but the height of its single absorption peak in sulfuric acid, at 305  $m\mu$ , indicated the presence of 30 mg. of material. This fraction was assumed to be a steroid because it was not found in the preinjection control fluid.

Another metabolite of cortisone, found opposite the corticosterone standard, gave a spectrum in sulfuric acid similar to that of corticosterone, but was shown not to consist of corticosterone. Evidence for the presence of  $\Delta^4$ -3-ketone and  $20,21\alpha$ -ketol groupings was obtained.

*Bioassays for Anti-Inflammatory Activity\**

Three eluates were tested for local activity in inhibiting granuloma formation, in a modified rat granuloma pouch test.<sup>9</sup> The only one possessing activity was the metabolite of medium mobility found three hours after injecting hydrocortisone. The known corticosteroids which have been found active in this test include a number of 20,21- $\alpha$ -ketols in addition to hydrocortisone and cortisone, so that a positive response does not necessarily reflect antirheumatic activity in man. Nevertheless, a new steroid, retaining activity in the granuloma test, appears to have been produced intra-articularly from hydrocortisone.

*Discussion*

It is evident that synovial tissue contains enzyme systems capable of producing a wide variety of alterations in the steroid molecule. The products ranged from corticosteroids more polar than those injected, through various stages of lesser oxygenation, to 17-ketosteroid material less polar than androsterone. Comparison of the metabolites of hydrocortisone found after one half-hour and three hours suggests that a sequence of metabolic reactions occurs. There also appears to be a degree of selectivity in the rates of absorption of various compounds from the synovial cavity since, in both three-hour experiments, the amounts of some metabolites exceeded those of the remaining injected steroids.

The rapid disappearance of the injected steroid from the synovial fluid raises the question as to whether the active hormone entered the blood unaltered, or whether extensive metabolism occurred in the lining membrane. Our finding of appreciable amounts of what was most probably hydrocortisone in the contralateral uninjected knee indicates that a large proportion of the injected hormone entered the circulation unchanged, and also escaped metabolism in the liver during the first 30 minutes post injection.

The difference between the anti-inflammatory activities of cortisone and hydrocortisone, when injected intra-articularly, remains to be explained. The simplest theory would be that cortisone must always be converted to hydrocortisone before becoming active, and that this conversion cannot be effected to a sufficient extent by synovial tissue. It is known that cortisone can be converted to hydrocortisone in the body.<sup>10</sup> Only a small proportion, however, is so metabolized, and numerous other metabolites are found in urine, yet cortisone has the same magnitude of activity as hydrocortisone when administered systemically in rheumatoid arthritis. Hence, it does not seem likely that cortisone acts through the formation of hydrocortisone.

It is also possible that both hormones must be transformed into another product (or products) which can be formed by synovial tissue from hydrocortisone, but not from cortisone. One form of evidence for this hypothesis is the fact that the intra-articular metabolites of cortisone were quite different from those of hydrocortisone. Had they formed the same metabolites, it

\* We are greatly indebted to Doctor Lewis H. Sarett of Merck and Company, Rahway, N. J., and to Doctor C. A. Winter of the Merck Institute of Therapeutic Research for performing these assays.

would have argued against the possibility of activity in a metabolite, since cortisone is locally inactive in the knee.

Similarly, cortisone may be ineffective in spite of its conversion to hydrocortisone in substantial amount, because the latter has not been further transformed, to a sufficient extent, to a requisite metabolite which accumulates in the knee. Hence, it is of interest to note that, three hours after intra-articular injection of hydrocortisone, one metabolite of medium mobility had accumulated in an amount almost twice that of the unchanged hydrocortisone itself, and that this material was active in the granuloma pouch test.

### Summary

The peripheral metabolism of cortisone and hydrocortisone in inflamed tissue has been investigated in order to gain insight into the mechanism of their anti-inflammatory activity. The following evidence for the local transformation of injected corticosteroid by rheumatoid synovial tissue has been obtained:

Chromatographic analysis of postinjection synovial fluid showed substantial amounts of a number of metabolites of intra-articularly injected cortisone and hydrocortisone. Only trace amounts were found when the uninjected contralateral knee was aspirated at the same time, indicating local metabolism, since, if metabolites had been formed elsewhere, they would have returned in more nearly equivalent concentrations to each side.

Both steroids yielded products of greater and lesser mobility, indicating that the tissue was capable of effecting a variety of metabolic transformations. A different pattern of metabolism was found for each steroid. The chief metabolites of cortisone (locally inactive) were hydrocortisone, several other corticosteroids, and an abundant noncorticosteroid component. Hydrocortisone (anti-inflammatory in the joint) gave rise to at least three other corticosteroids,  $11\beta$ -hydroxy- $\Delta^4$ -androstene-3,17-dione, and several other 17-ketosteroids. The difference in metabolic pattern, together with the presence of relatively large amounts of hydrocortisone following cortisone injection, raises the question as to whether the unique anti-inflammatory effect of hydrocortisone in the synovial cavity may not reside in its transformation to a specific metabolic product.

### References

1. WILSON, H., J. GLYN, E. SCULL, C. McEWEN & M. ZIFF. 1953. Rate of disappearance and metabolism of hydrocortisone and cortisone in the synovial cavity in rheumatoid arthritis. *Proc. Soc. Exptl. Biol. Med.* **83**: 648.
2. WILSON, H., & R. FAIRBANKS. 1954. Chromogenic values of 17-hydroxy corticosteroids in a modified Porter-Silber reaction. *Arch. Biochem. Biophys.* **53**: 71.
3. ZACCO, M., E. M. RICHARDSON, J. O. CRITTENDEN, J. L. HOLLANDER & F. C. DOHAN. 1954. Disposition of intra-articularly injected hydrocortisone and cortisone acetate in rheumatoid arthritis. I. Concentration in synovial fluid and cells. *J. Clin. Endocrinol. and Metabol.* **14**: 711.
4. GALLAGHER, T. F., L. HELLMAN, H. L. BRADLOW, J. ZUCKNER & R. FREYBERG. 1953. Dynamics of radioactive cortisone distribution in rheumatoid arthritis. *Proc. Ann. Meet., 1953, of the A.R.A. Ann. Rheumatic Diseases.* **12**: 347.
5. WILSON, H. & R. FAIRBANKS. 1955. A rapid micromethod for the determination of 17-hydroxy- and 17-ketosteroids. *Arch. Biochem. Biophys.* **54**: 440.

6. BURTON, R. B., A. ZAFFARONI & E. H. KEUTMANN. 1951. Paper chromatography of steroids: II Corticosteroids and related compounds. *J. Biol. Chem.* **188**: 763.
7. BERNSTEIN, S. & R. H. LENHARD. 1953. The absorption spectra of steroids in concentrated sulfuric acid. I. Method and data. *J. Org. Chem.* **18**: 1146.
8. BURSTEIN, S., E. NADEL & R. DORFMAN. 1954. A new steroid in human urine. *Arch. Biochem. Biophys.* **53**: 307.
9. MEIER, R., W. SCHULER & P. DESAULLES. 1950. Zur Frage des Mechanismus der Hemmung des Bindegewebswachstums durch Cortisone. *Experientia.* **6**: 469.
10. BURTON, R. B., E. H. KEUTMANN & C. WATERHOUSE. 1953. The conversion of cortisone acetate to other alpha-ketolic steroids. *J. Clin. Endocrinol. and Metabol.* **13**: 48.



# THE USE OF INTRA-ARTICULAR HYDROCORTISONE, ITS ANALOGS, AND ITS HIGHER ESTERS IN ARTHRITIS\*

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Exactly four years ago, on Jan. 27, 1951, our study group administered the first intra-articular hydrocortisone injections into the knees of three patients with rheumatoid arthritis. Within 24 hours, the palliation of the local inflammation became obvious, both clinically, and by the drop toward normal in the intra-articular temperature of the injected knees.<sup>1</sup>

Since this time, we have injected hydrocortisone into the joints, bursae, or tendon sheaths of nearly 1500 patients nearly 24000 times for the local alleviation of inflammation from a variety of conditions. Our results from this form of local therapy have been previously reported.<sup>2-5</sup> Sixty-seven additional reports by others in the American and foreign medical literature attest the widespread acceptance of this local palliative therapy.<sup>6</sup>

Intra-articular hydrocortisone produces a temporary amelioration in about 80 per cent of arthritic joints but, in one third of these, the effect persists only for a day or two, precluding any practical value as treatment. Thus, the repeated intra-articular injection of hydrocortisone acetate has been of continued therapeutic value in only slightly more than half of the arthritic patients. A search has been conducted, therefore, to find an agent capable of increasing and prolonging the local effect.

Our previous studies<sup>7, 8</sup> on the disappearance of hydrocortisone acetate from the synovial cavity after injection have shown that much of the hormone is absorbed into the synovial membrane and is apparently stored there. Furthermore, this absorbed hydrocortisone acetate remains for some days in the synovial tissue as unchanged hydrocortisone ester, rather than as the metabolites found in the synovial fluid within a few hours after injection. We assumed from this finding that higher esters of hydrocortisone might likewise be absorbed and stored unchanged and, being less soluble, might prolong the effectiveness.

Such higher esters and some analogs, of hydrocortisone were prepared for us in the laboratories of Merck and Co. The comparative results on all 12 preparations are to be reported here.

## *Method*

Comparative assay of the various preparations has been conducted by the substitution of each new suspension for the hydrocortisone acetate usually instilled into the arthritic joint. Except in the case of 9- $\alpha$ -fluoro-hydrocortisone, doses of the "unknown" identical to the previous dose of hydrocortisone acetate (usually 37.5 mg.) were injected into the arthritic knee joint

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under study. Patients were all unaware of the substitution. Follow-up examinations were made to determine the degree of subsidence of joint symptoms and signs, and for duration of the effect. Throughout the study period, the patients kept diaries of symptomatic effects from all injections. Whenever comparative effects were equivocal from a new preparation, the injection of the acetate ester was repeated, and the "unknown" was again tried for the next reinjection. To avoid cumulative effects, injections were never repeated oftener than at two-week intervals.

The following analogs were compared with hydrocortisone acetate for degree of anti-inflammatory effect, and for duration of effectiveness: hydrocortisone (free alcohol), 9-alpha-chloro-hydrocortisone acetate, 9-alpha-fluoro-hydrocortisone acetate, allo-dihydro-hydrocortisone, and 14-hydroxy-hydrocortisone. The following esters of hydrocortisone were subjected to comparative assay: hydrocortisone tertiary-butyl acetate, hydrocortisone caprylate, hydrocortisone benzoate, hydrocortisone palmitate, hydrocortisone trimethyl hexanoate, and hydrocortisone methyl neopentyl acetate.

### *Results*

The first comparative study of intra-articular effectiveness utilized the hydrocortisone t-butyl acetate. Our previously reported results in 171 cases<sup>6</sup> showed that in 65 per cent of arthritic joints the higher ester, injected in identical dose with that previously used for the acetate, produced more complete palliation of symptoms and signs. In 30 per cent, there was no difference in effect and, in 5 per cent, the acetate had a superior anti-inflammatory effect.

The duration of effectiveness was much more easily quantitated. When the number of days' duration of effect from injection of the t-butyl acetate ester was compared with the duration of effect from the acetate ester, it was found that, in 59 per cent, the newer ester had a significantly longer palliative effect. In 35 per cent, the difference in duration was insignificant and, in 6 per cent, the acetate effect was somewhat longer than that from the t-butyl acetate.

While there was considerable difference from patient to patient in the duration of effect from either of the preparations, the effects in the same case from reinjection of the same preparation were fairly consistent both in degree of palliation and in duration. By comparing the total number of days of relief produced following one injection each in the 171 cases, we found that the hydrocortisone t-butyl acetate almost doubled the average duration. In a few cases, the effect was prolonged as much as 10 times over that previously experienced from hydrocortisone acetate intra-articularly.

In an effort to facilitate finding still more effective preparations for intra-articular use, we chose 17 patients who had rheumatoid arthritic knees which had been repeatedly injected with hydrocortisone acetate. The degree and duration of effect in these cases had varied considerably from one to the other, but in each case they were consistent from one injection to the next. They form the basis for comparison of all the other analogs and esters of hydrocortisone studied, the preparations being substituted for one another in differing sequence.

TABLE 1

COMPARATIVE DURATION OF ANTI-ARTHRITIC EFFECTIVENESS FROM INTRA-ARTICULARLY  
INJECTED ESTERS OF HYDROCORTISONE  
(Duration expressed in days of maximal effect)

Case	F A	F t-BA	F Cap.	F Benz.	F Palm.	F Hex.	F Pent.
a	4	9	3	—	6	3 (r)	4 (r)
b	10	9	3	11 (r)	9 (r)	5	2
c	6	6	6	—	10	4	1 (r)
d	2	6	1	—	3	2	2
e	14	14	14	3	8	8	5
f	7	14	16	—	5	2 (r)	6
g	8	13	—	10	9	8	7
h	2	4	4	—	2	0	1
i	7	12	8	14	4	7	6
j	10	30	13	—	16	—	—
k	2 (r)	11	5 (r)	1 (r)	—	3	1 (r)
l	4	14	—	4 (r)	2	—	2
m	5	7	18	—	8	6	14
n	2	7	2	—	—	3	—
o	5	14	0	14	7	9	10
p	8	8	8	7	2	6	5
q	9	12	0	7 (r)	10	8	11
Average.....	6	12	7	8	7	5	5
Duration quotient.....	1	2.0	1.16	1.3	1.16	0.8	0.8

(r) = reaction — temporary exacerbation following injection.

TABLE 2

COMPARATIVE DURATION OF ANTI-ARTHRITIC EFFECTIVENESS OF INTRA-ARTICULARLY  
INJECTED ANALOGS OF HYDROCORTISONE  
(Expressed in days of maximal effect)

Case	F A	F OH	9-a-chlor F	9-a-fluor F*	Allo-dihy F	14-OH F
a	4	4	6	5	0	2
b	10	7	7	4	0	3
c	6	4 (r)	6	3 (e)	0	2 (r)
d	2	2	7	2 (e)	0	0
e	14	9	11	8	0	4
f	7	7	8	5 (e)	0	
g	8	6	10	8	0	5
h	2	3	4	4	2	
i	7	6	9	7 (e)	0	3
j	10	8	9	3	0	
k	2 (r)	4	7	5 (e)	6	
l	4	5	11	9	0	5
m	5	5	6	4 (e)	0	
n	2		2	4 (e)	2	1
o	5	4	9	4	1	
p	8	8	7		1	3
q	9	7	8	0 (e)	1	
Average duration.....	6	5.5	8	4.7*	0.8	2.8
Duration quotient.....	1	0.9	1.3	0.78*	0.13	0.47

\* Dose only 5 mg.; all others, 37.5 mg.

(r) = reaction — temporary exacerbation following injection.

(e) = edema noted in treated leg after injection.

No figures could be reached to quantitate the relative degree of anti-inflammatory effectiveness in these cases from the various preparations, but the duration of effect was easily quantitated, and is presented on TABLES 1 and 2.

From TABLE 1 the wide variation in individual response from any of the forms studied is evident, but the totals showing the average number of days' relief from each preparation give us definite figures for comparison. Thus, it appears that hydrocortisone t-butyl acetate (F t-BA) produces local palliation averaging 12 days, as compared with 6 days on the average from hydrocortisone acetate (F A). Enhancement of the duration of effect, as compared with the standard F acetate from the other esters was not considered significant. By dividing the average days' relief afforded by use of each newer ester by 6 (the average duration of effect from hydrocortisone acetate) we have obtained a

### INTRA-ARTICULAR PALLIATION OF RHEUMATOID ARTHRITIS

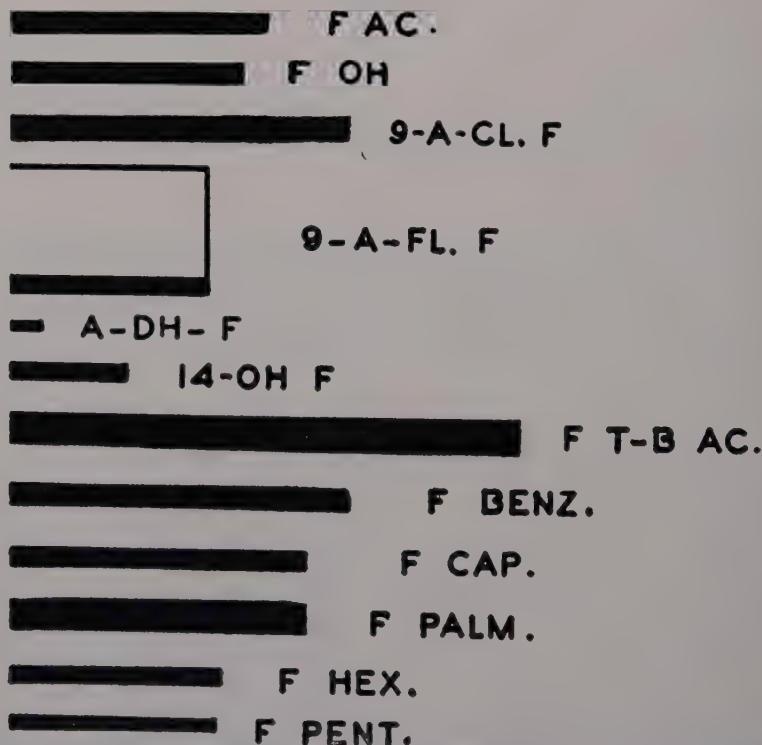


FIGURE 1. Relative anti-inflammatory effectiveness of analogs and esters of hydrocortisone by intra-articular injection into rheumatoid arthritic joints. (Length of blocks = relative duration of palliative effect, height of blocks = relative degree of effect.) F AC. = hydrocortisone acetate. F OH = hydrocortisone (free alcohol). 9-A-CL.F = 9- $\alpha$ -chloro hydrocortisone. 9-A-FL.F = 9- $\alpha$ -fluoro hydrocortisone. A-DH-F = allo-dihydrohydrocortisone. 14-OH F = 14-hydroxy hydrocortisone. F T-B AC. = hydrocortisone t-butyl acetate. F BENZ. = hydrocortisone benzoate. F CAP. = hydrocortisone caprylate. F PALM. = hydrocortisone palmitate. F HEX. = hydrocortisone trimethyl hexanoate. F PENT. = hydrocortisone methyl neopentyl acetate.



"duration quotient." It will be noted that the F benzoate, F hexanoate, and F neopentyl acetate were somewhat irritating, since a greater incidence of postinjection reactions occurred with their use.

The same method was used in TABLE 2 for the comparison of duration of effect from analogs of hydrocortisone. None of those studied was effective in producing significantly longer palliation than hydrocortisone acetate, and the allo-dihydro-hydrocortisone and 14-hydroxy-hydrocortisone were considerably inferior.

The 9- $\alpha$ -fluoro-hydrocortisone acetate in doses of only 5 mg. produced almost as marked an amelioration, and the effect lasted almost as long as 37.5 mg. of hydrocortisone acetate, proving the increased potency already reported by others using this analog systemically. In half of the patients, however, this injection led to edema of the injected leg, which persisted several days. The mechanism involved in production of this local edema is still under study.

In FIGURE 1 we have plotted, to scale, the comparative degree and duration of palliation from these various preparations. The length of each block represents relative duration of the average effect, and the height of each, the comparative degree of palliation from each preparation. The actual degree of effect from 5 mg. of 9- $\alpha$ -fluoro-hydrocortisone is surmounted by a space representing the theoretical proportion of effect if an equal dose (37.5 mg.) had been possible. It is doubtful, however, that a higher dose would increase *duration* of effect, since increasing the intra-articular dose has seldom appreciably increased duration of effect using other forms of hydrocortisone.

This chart, therefore, is most important as a summary of relative duration of anti-inflammatory effectiveness, and it can be seen at a glance that the hydrocortisone t-butyl acetate is the most effective agent studied thus far. Preparations of 9- $\alpha$ -fluoro-hydrocortisone t-butyl acetate are being made for further studies. Metacortandralone and metacortandracin are also being studied by intra-articular injection.

### Conclusions

(1) Through four years of clinical experience, intra-articular hydrocortisone has proved a valuable adjunct in the local treatment of rheumatic diseases.

(2) As a result of intra-articular injection of 12 different analogs or esters of hydrocortisone, it appears that the tertiary-butyl acetate ester is most effective in prolonging the local palliative effect in rheumatoid arthritic joints. The mechanism of this increased effect has not yet been determined.

(3) The increased anti-inflammatory potency of 9- $\alpha$ -fluoro-hydrocortisone has been confirmed by this rapid method of local assay, but the production of edema of the injected leg in one half the cases nullified the advantage gained by the increased potency, and the duration of the local palliation was not enhanced.

(4) The search continues for more effective preparations. The ideal anti-inflammatory hormone preparation for intra-articular use should be effective in small dosage and should produce no adverse effects but, particularly, it should have a *prolonged* local action so that reinjections given for continued palliation of chronic arthritis may be spaced widely.

*References*

1. HOLLANDER, J. L., E. M. BROWN, R. A. JESSAR & C. Y. BROWN. 1951. Hydrocortisone and cortisone injected into arthritic joints. *J. Am. Med. Assoc.* **147**: 1629.
2. BROWN, E. M., J. B. FRAIN, L. UDELL & J. L. HOLLANDER. 1953. Locally administered hydrocortisone in the rheumatic diseases. *Am. J. Med.* **15**: 656.
3. HOLLANDER, J. L. 1953. Intra-articular hydrocortisone in arthritis and allied conditions. *J. Bone Joint Surg.* **35**(A): 983.
4. HOLLANDER, J. L. 1953. Intra-articular hydrocortisone in the treatment of arthritis. *Ann. Internal Med.* **39**: 735.
5. HOLLANDER, J. L., E. M. BROWN & R. A. JESSAR. 1954. Intra-articular hydrocortisone in the rheumatic diseases. *Med. Clinics N. Amer.* **38**: 349.
6. HOLLANDER, J. L., E. M. BROWN, R. A. JESSAR, L. UDELL, N. M. SMUKLER & M. A. BOWIE. 1954. Local anti-rheumatic effectiveness of analogues and higher esters of hydrocortisone (with complete bibliography on intra-articular hydrocortisone). *Ann. Rheumatic Diseases.* **13**: 285.
7. HOLLANDER, J. L. 1953. Discussion, *Proc. Am. Rheumatism Assoc. Ann. Rheumatic diseases.* **12**: 347.
8. ZACCO, M., E. M. RICHARDSON, J. O. CRITTENDEN, J. L. HOLLANDER & F. C. DOHAN. 1954. Disposition of intra-articularly injected hydrocortisone acetate, hydrocortisone, and cortisone acetate in arthritis. *J. Clin. Endocrinol. Metabol.* **14**: 711.

*Discussion of the Paper*

QUESTION: Do you have any information as yet on the local effects of Meticorten?

DOCTOR HOLLANDER: Our preliminary results with Meticorten injected intra-auricularly have been almost completely negative. Meticortelone, on the other hand, shows definite activity by intra-auricular injection. Results will be reported in full later. Since Meticorten is an analog of cortisone, it is not surprising that it has little local effect in the joint, whereas Meticortelone, being an analog of hydrocortisone, is active.

## Part IV. The Topical Use of Hydrocortisone

### A. SKIN

#### FAILURE TO DEMONSTRATE ABSORPTION OF HYDROCORTISONE WHEN TOPICALLY APPLIED TO THE HUMAN SKIN

By C. Conrad Smith

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Along with the favorable reports of systemically administered cortisone, ACTH, and hydrocortisone in many dermatoses<sup>1-3</sup> and other diseases were also observations of certain untoward physiologic effects from these hormones. Consequently, the investigation of the local application of these hormones directly on skin lesions represented a logical attempt to obviate the systemic ill effects. The topical application of cortisone in skin disease was found to be ineffective,<sup>4, 5</sup> with the possible exception of certain skin eruptions on sites immediately surrounding the mucocutaneous junctions. By contrast, topically applied hydrocortisone was found to have favorable effect in a number of dermatoses.<sup>6-11</sup> Some observers felt that a hypothetical explanation for the effectiveness of topically applied hydrocortisone by comparison with the ineffectiveness of topically applied cortisone was the possible greater percutaneous absorption of the former. The previously demonstrated apparent lack of systemic absorption from topically applied cortisone<sup>12</sup> was in favor of this theory at the time.

#### *Review of Methods*

Using the circulating eosinophile count<sup>13</sup> as an indication to percutaneous absorption or nonabsorption of hydrocortisone,<sup>14</sup> just as Danto and Maddin<sup>12</sup> had done in their studies on cortisone ointment, the following experiment was done (a reduction in this count as a test for absorption was the most practical method available at the time).

Base line (preinunction) circulating eosinophile counts were done on eight normal human volunteers and seven hospitalized patients with generalized skin disease. Each of the 15 subjects received inunctions with 6 gm. of ointment containing a total 150 mg. of hydrocortisone acetate. Circulating eosinophile counts were done at 4, 6, and 28 hours after the inunction, at the same hours of the day that the baseline counts were done.

A control study was done in the same manner as just outlined with the ointment base alone.

No constant alteration by comparison with the control study was demonstrated in the circulating eosinophile count before and after the inunction of the ointment containing 150 mg. of hydrocortisone in normal skin areas of human volunteers or in affected skin of hospitalized patients with generalized skin disease.

Witten, Shapiro, and Silber<sup>15</sup> recently completed further studies on the absorption of topically applied hydrocortisone using a new and more sensitive method. Normal skin of 6 human volunteers and affected skin of 9 patients with generalized skin disease received inunctions with 10 gm. of ointment containing 25 mg./gm. of hydrocortisone acetate daily for 3 days until a total of 30 gm. of ointment and 750 mg. of hydrocortisone acetate was applied. Utilizing the Silber-Porter method,<sup>16</sup> these investigations were unable to demonstrate evidence of systemic absorption.

Further experiments<sup>17</sup> now in process of completion were to ascertain changes, if any, in the urinary 17-ketosteroids and 17-hydroxycorticosteroids after inunction with hydrocortisone ointment. A suppression of the urinary 17-ketosteroids and an increase in the 17-hydroxycorticosteroids was the expected finding in the event of demonstrable systemic absorption. Baseline (preinunction) 24-hour urine specimens were obtained on normal male subjects and quantitative assays made for 17-ketosteroids and 17-hydroxycorticosteroids. The same assays were done on 24-hour urine specimens after inunction with 10 gm. of ointment containing a total of 250 mg. of hydrocortisone (free alcohol).

As a control, the same experiment is being repeated on the same persons, in the manner just outlined but with inunction of the ointment base alone.

Though not complete, the results thus far are in consonance with that of the two aforementioned studies.

### *Summary and Conclusion*

Three investigative studies, directed at demonstrating evidence of systemic absorption from topically applied hydrocortisone, have been reviewed. In each study, no systemic absorption was demonstrated.

In the first and last discussed experimental study, the results indicate that either there was a lack of systemic absorption or insufficient absorption to produce a drop in the circulating eosinophile count or any alteration in the urinary steroids, respectively. As pointed out by Witten *et al.* in their study, however, the experimental lack of evidence to demonstrate percutaneous absorption of hydrocortisone "does not rule out the possibility that the hydrocortisone is converted to still another compound, or so bound with other molecules as not to be detectable by the method used."

This experimental evidence of nonabsorption of topically applied hydrocortisone fits in with the clinical observations of the many patients who have used hydrocortisone ointment in quantity over prolonged periods, without a single instance of systemic effects.

### *References*

1. SULZBERGER, M. B., V. H. WITTEN & S. N. YAFFE. 1951. Cortisone acetate administered orally in dermatologic therapy. *Arch. Dermatol. and Syphilol.* **64**: 573.
2. KIERLAND, R. R., P. A. O'LEARY, L. A. BRUNSTING & J. W. DIDCOCK. 1952. Cortisone and corticotropin (ACTH) in dermatology. *J. Am. Med. Assoc.* **148**: 23.
3. HOPKINS, J. G., B. M. KESTEN, C. T. NELSON, G. W. HAMBRICK, R. G. JENNINGS & G. F. MACHACEK. 1952. Pituitary adrenocorticotrophic hormone (ACTH) and cortisone in diseases of the skin. *Arch. Dermatol. and Syphilol.* **65**: 401.
4. GOLDMAN, L., R. G. THOMPSON & E. R. TRICE. 1952. Cortisone acetate in skin dis-



- ease: local effect in the skin from topical application and local injection. *Arch. Dermatol. and Syphilol.* **65**: 177.
5. SULZBERGER, M. B. 1952. Some aspects of ACTH and cortisone in dermatology. 10th Intern. Congr. Dermatol., London, England. *Excerpta Med.* **13**. *Dermatol. & Venereol.* **19**: 101.
  6. SULZBERGER, M. B. & V. H. WITTEN. 1952. The effect of topically applied compound F in selected dermatoses. *J. Investigative Dermatol.* **19**: 101.
  7. SULZBERGER, M. B., V. H. WITTEN & C. C. SMITH. 1953. Hydrocortisone (compound F) acetate ointment in dermatological therapy. *J. Am. Med. Assoc.* **151**: 468.
  8. SULZBERGER, M. B., V. H. WITTEN & C. C. SMITH. 1953. Hydrocortisone (compound F) free alcohol ointment in dermatological therapy. *J. Am. Med. Assoc.* **152**: 456.
  9. SIDI, E., J. BOURGEOIS-CAVARDIN & O. PLAS. 1953. Topical application of hydrocortisone acetate in treatment of eczema and pruritus. *Presse méd.* **61**: 992.
  10. SULZBERGER, M. B. & V. H. WITTEN. 1954. Hydrocortisone ointment in dermatological therapy. *Med. Clinics N. Amer.* **38**: 321.
  11. ROBINSON, H. M. & R. C. V. ROBINSON. 1954. Treatment of dermatoses with local application of hydrocortisone acetate. *J. Am. Med. Assoc.* **155**: 1213.
  12. DANTO, J. L. & S. MADDIN. 1952. The eosinophilic response in normal subjects following the inunction of cortisone ointment. *J. Investigative Dermatol.* **18**: 381.
  13. THORN, G. W., A. E. RENOLD, D. L. WILSON, T. F. FRAWLEY, D. JENKINS, J. GARCIA-REYS & P. M. FORSHAM. 1951. Studies on the activity of orally administered cortisone. *New Engl. J. Med.* **245**: 549.
  14. SMITH, C. C. 1953. Eosinophilic response after inunction of hydrocortisone ointment. *Arch. Dermatol. and Syphilol.* **68**: 50.
  15. WITTEN, V. H., A. J. SHAPIRO & R. H. SILBER. 1955. Attempts to demonstrate absorption of hydrocortisone by new chemical test following inunction into human skin. *Proc. Soc. Exptl. Biol. Med.* In press.
  16. SILBER, R. H. & C. C. PORTER. The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma. *J. Biol. Chem.* In press.
  17. SMITH, C. C. Urinary excretion of 17-ketosteroids and 17-hydroxycorticosteroids after inunction of hydrocortisone ointment. *J. Invest. Dermatol.* In press.

# HISTOLOGICAL EFFECTS OF HYDROCORTISONE IN THE SKIN OF MAN\*

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An interesting and important phase of the current studies on the corticosteroids is the observation of their local reactions directly in tissue. This study in the easily available skin of man provides for some morphologic detail of analysis of these reactions at the cellular level. Moreover, these local reactions have the additional value of being important parts of technique for assay of new corticosteroids, which may have local activity in the tissues of man.<sup>1</sup>

Throughout the past three years, we have been studying these reactions in normal and pathologic skin.<sup>2, 3</sup> Because of the difficulty of carrying over the analogy of the tissue reactions in the skin of animals, we have confined our studies to the skin of man. The technique of study, in brief, is the analysis of the skin after local injection of varying concentrations of corticosteroids into superficial portion of the skin, and also after local application to the surface of the skin. Additional studies have also been done with deeper injection by means of the Hypospray Jet Injection Apparatus.

The histological studies have included the examination of the skin site after local injection of the crystals of the steroid. These reactions have been studied locally in the tissue by means of routine histological technique and special histochemical studies. Additional studies have been done by excising the complete area of injection and submitting this material for colorimetric and chromatographic assays.

In normal skin, the relatively insoluble crystals of the corticosteroids may be seen especially with frozen section techniques, and in paraffin sections with the paraffin removed with xylene and alcohols above 70 per cent, and examined in detail under polarizing light, especially with the variable color polarizing filters. Attempts to color the crystals have not been successful. There is no apparent increase in the size of the crystal deposited in tissue, but there may be fragmentation by the technique of injection. Studies of the mass of these crystals after varying time intervals have not yielded any significant facts as regards method of solution of these crystals, especially about the periphery of the mass, or any changes in physical character. Crystals, especially of the hydrocortisone acetate, have been persistent for months. The crystals of the free alcohol have disappeared in a period of 7 to 10 days. The persistency of the crystalline masses of the 9 $\alpha$  fluorohydrocortisone acetate and free alcohol is still under study. The free alcohol of 9 $\alpha$  fluorohydrocortisone appears to be more persistent in tissue than the free alcohol of hydrocortisone. These studies of persistency in tissue parallel, of course, the tissue solubility of these materials.

A characteristic feature following the local injection of cortisone, hydrocortisone, and its analogs has been the rapid appearance in formaldehyde fixed paraffin sections of a granular type of hematoxylinophilic masses occurring

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in the site of deposition of the crystals. These masses have been found with hydrocortisone and the fluorohydrocortisones. There is very little inflammatory reaction about these bluish granular masses. The connective tissue cells covered with these crystals may show pyknosis and karyorrhexis. Attempts have been made to analyze the exact character of these hematoxylinophilic masses. Detailed histochemical studies are still under way with Doctor William Atkinson<sup>4</sup> and Doctor Don Opdyke.<sup>5</sup> In brief, at present, the following characteristics have been determined for these masses: (1) much more intense appearance with formaldehyde fixation; (2) periodic Schiff positive, weak Feulgen; (3) metachromasia; (4) no change with diastase digestion; (5) no change with ribonuclease; (6) no change with desoxyribonuclease; (7) no change with hyaluronidase early, later may take out some of the Alcian blue reaction; (8) early no free lipids in the site with Sudan black or Oil red O, later, lipids, especially in histiocytes; (9) elastase, no change; and (10) minimal inflammatory reaction. The 9 $\alpha$  fluorohydrocortisone acetate and free alcohol show somewhat different types of hematoxylinophilic masses, but the same histochemical reactions. It is assumed, without any definite proof, that a large part of this granular mass may be related to changes in ground substance plus the additional activity of some cellular destruction. Additional studies are being carried out on the characteristics of these masses, both in normal and pathologic skin.

These masses remain in tissue as long as the associated crystal depot is present. Later, they are broken up and fragmented, and removed by histiocytes containing lipids. A temporary type of skin "atrophy" of a poikilodermatous type is found. There have been no permanent sequelae. The longest period of histological observation after local injection has been four months. Injected areas, however, have been examined clinically after more than a year and have shown no abnormalities. So far, in normal skin, after two months, injections of 9 $\alpha$  fluorohydrocortisone acetate and free alcohol show none of this type of "atrophy."

In pathologic skin, the masses appear to be removed more rapidly ("the demand response of inflammation"). As compared with the control areas of inflammation, the areas into which the hydrocortisone have been injected reveal: (1) less edema; (2) less inflammatory infiltrate; (3) later, a decrease in acanthosis, if acanthosis had been a feature of the dermatitis; (4) some tendency to perivascular collection; and (5) lymphocytolysis. Where the inflammatory reaction is very severe, no clinical recognizable inhibition of inflammation has occurred. In such sections, however, there will be some evidence, locally, about the deposition of the crystals of the hydrocortisone. The greatest number of studies of pathologic skin have been done with psoriasis, and this phase has been included in details of bio-assay techniques, because of the uniformity in response to local injections.<sup>1</sup> In the study of pathologic skin, hydrocortisone acetate has been used, for the most part, because of its persistency in tissue.

In the field of tumors, the local injections have been found to be most active locally in leukemia and mycosis fungoids. Lymphocytolysis has been evident in these conditions.

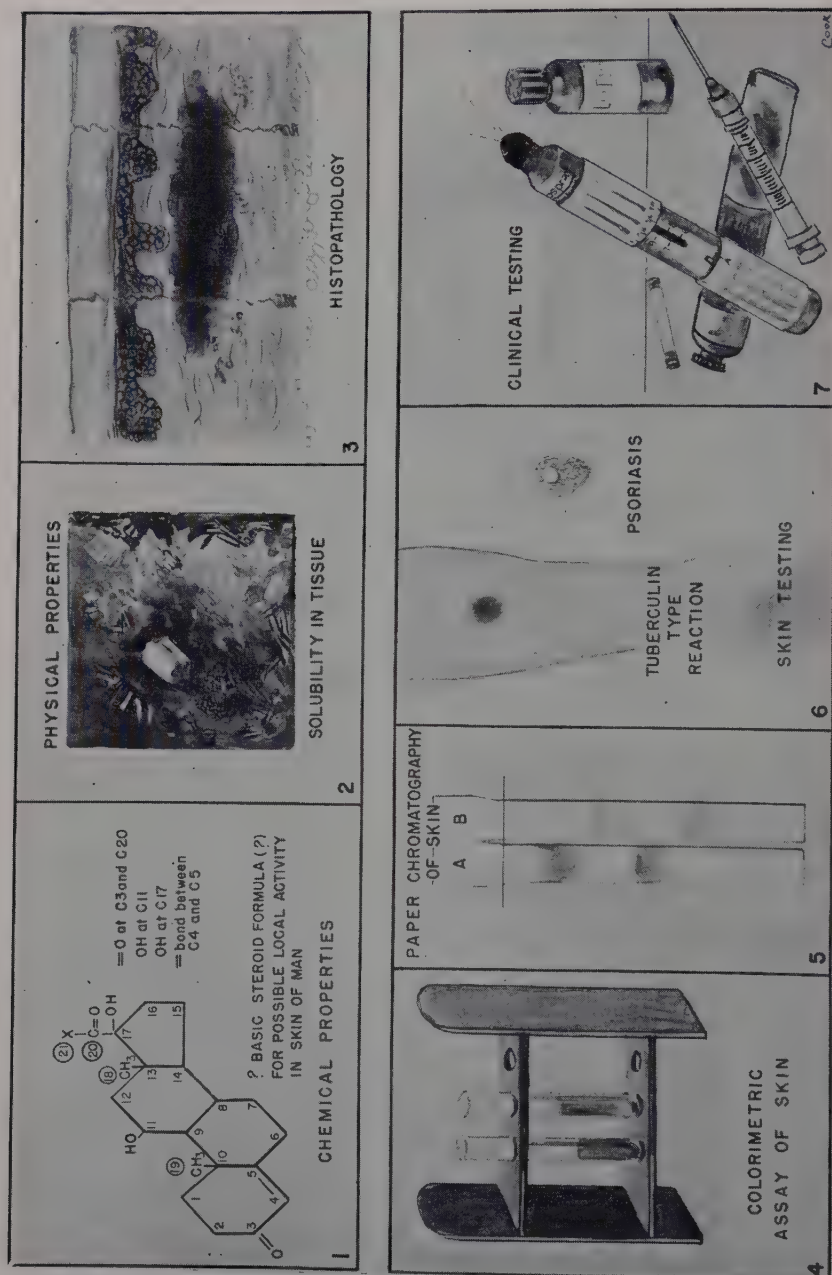


FIGURE 1. Showing phases of an assay technique for a corticosteroid having local activity in the skin of man and the importance of the histopathology in this technique (from Goldman, Flat, and Basketl).





FIGURE 2. Hematoxylinophilic masses after local injection of hydrocortisone acetate 48 hours.  $\times 160$  (hematoxylin-eosin).

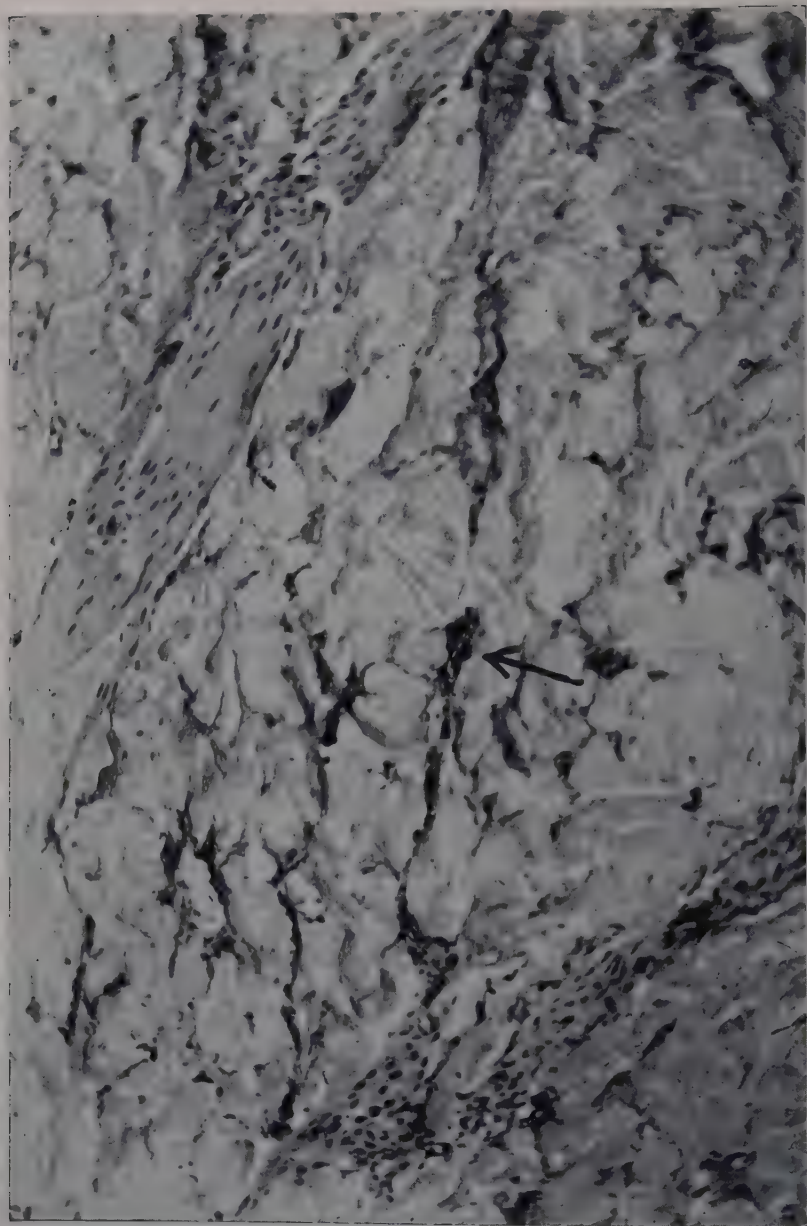


FIGURE 3. Hematocolinophilic masses after local injection of  $9\alpha$  fluorohydrocortisone acetate 48 hours.  $\times 160$  (hematoxylin-eosin).

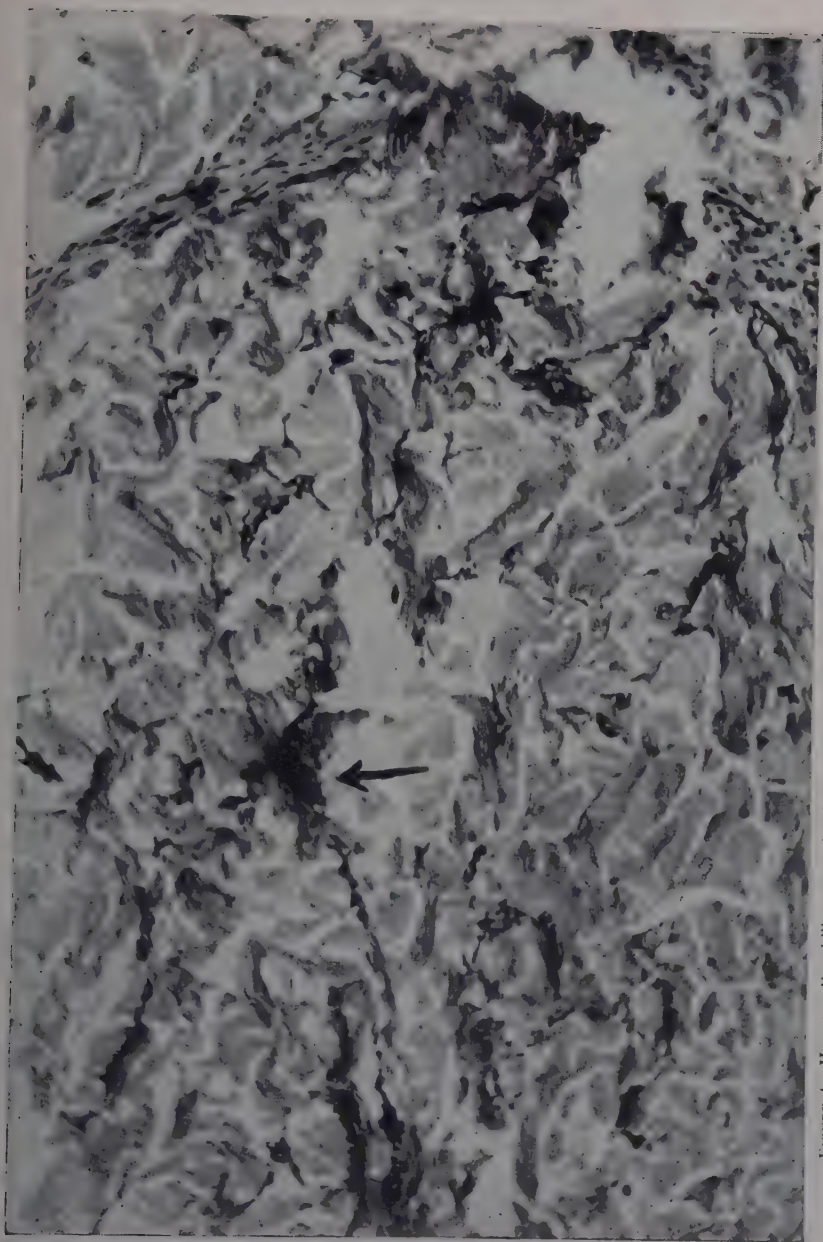


FIGURE 4. Hematoxylinophilic masses after local injection of 9 $\alpha$  fluorohydrocortisone free alcohol 5 days.  $\times$  160 (hematoxylin-eosin).



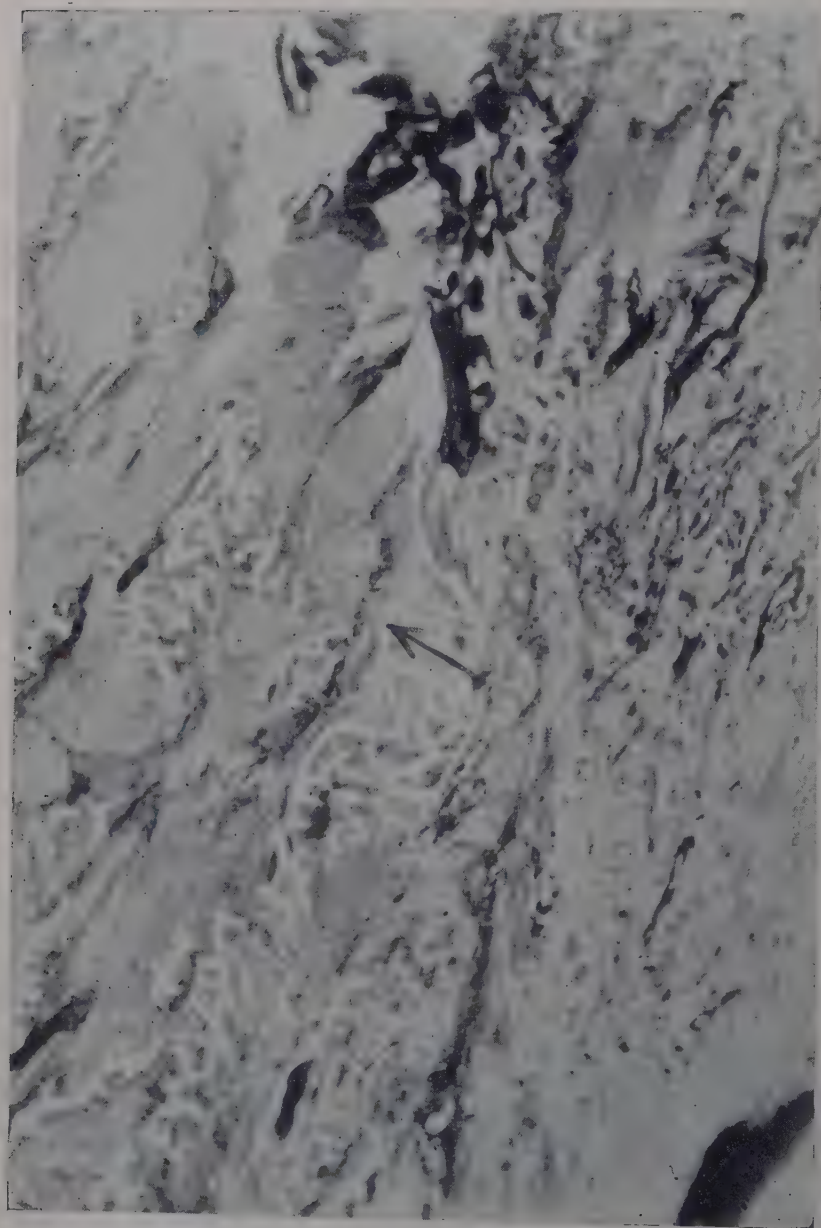


FIGURE 5. Metachromasia with toluidine blue pH 4.5 48 hours.  $\times 160$ .



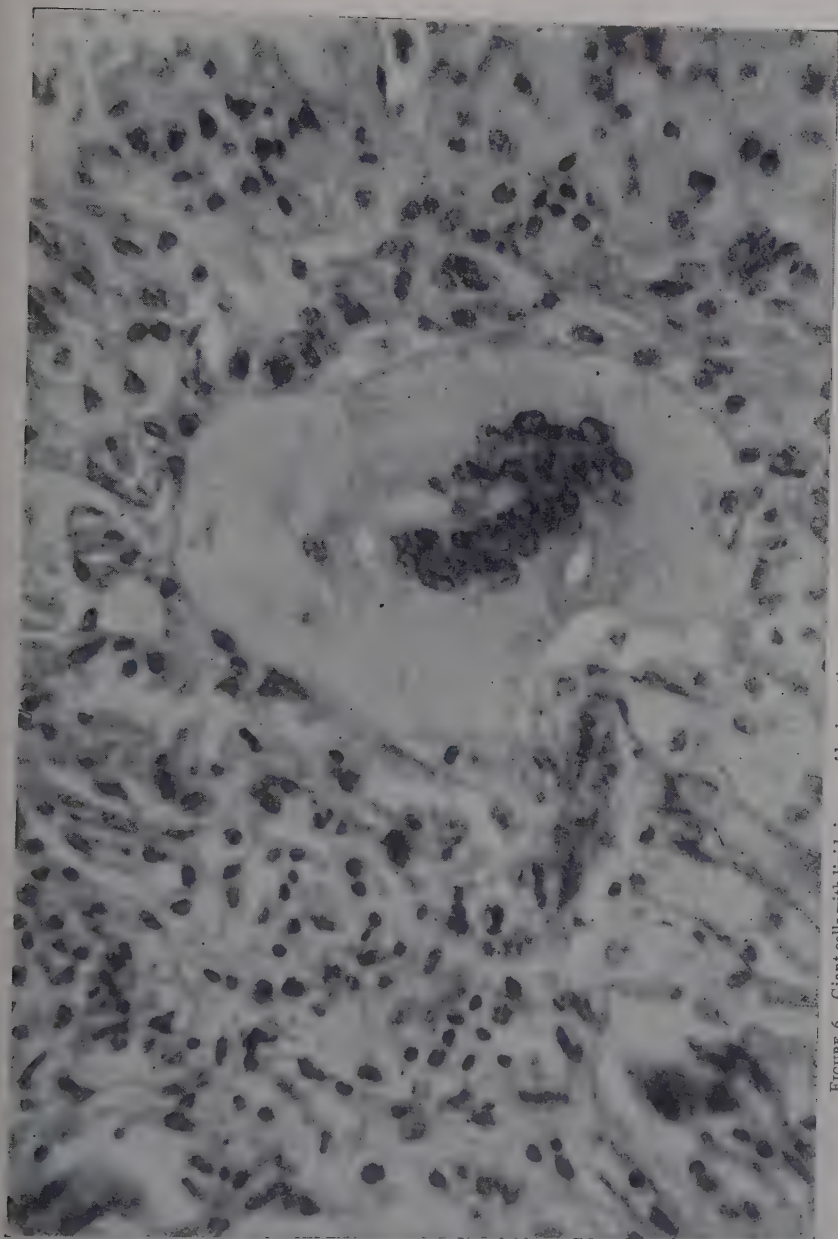


FIGURE 6. Giant cells with lipids in area of hydrocortisone acetate 111 days after injection.  $\times 250$  (hematoxylin-eosin).

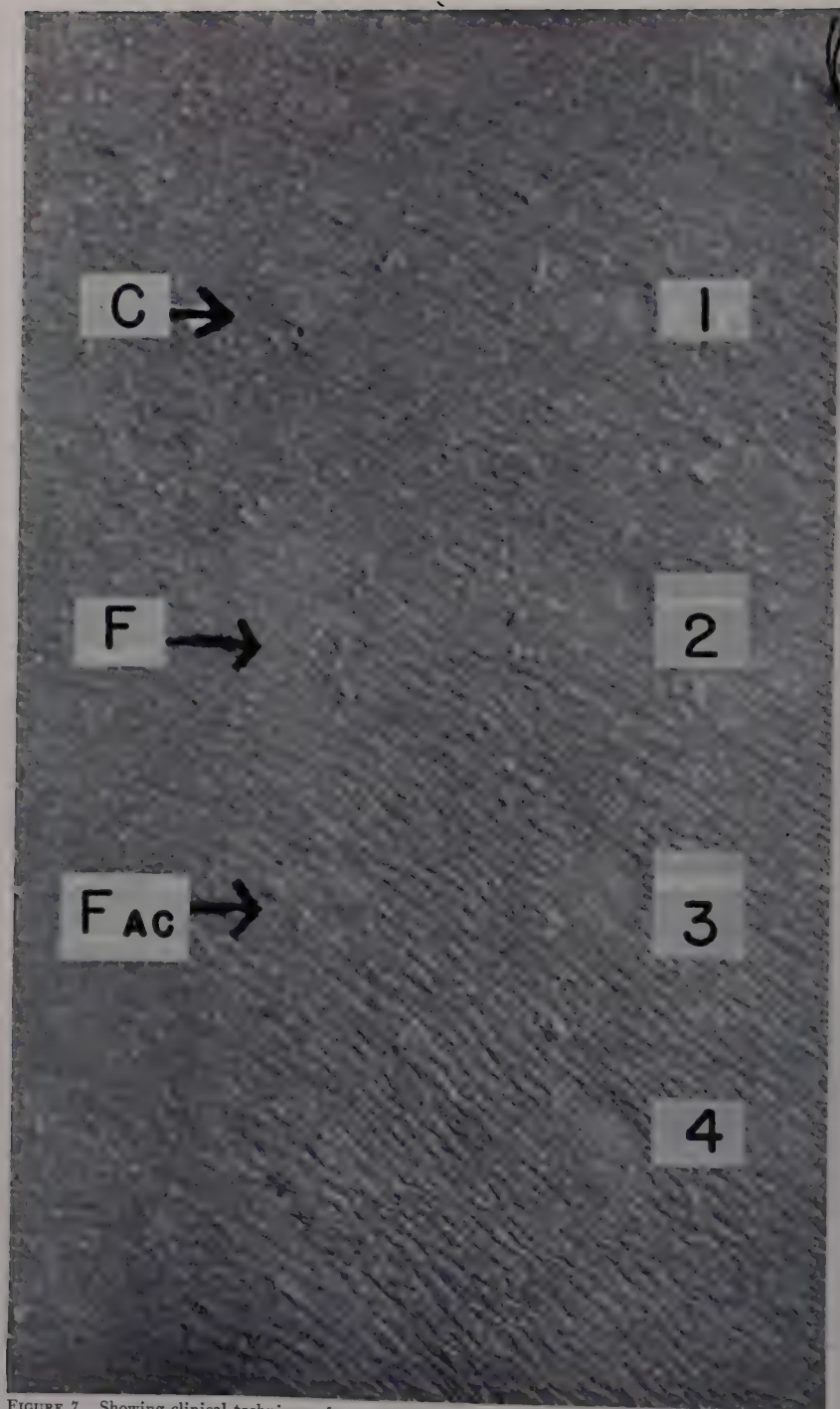


FIGURE 7. Showing clinical technique of assay in psoriasis following local injections C (5 mg. cortisone) F (5 mg. hydrocortisone free alcohol) F AC (hydrocortisone acetate 5 mg.) 1 (hydrocortisone acetate 5 mg.) 2 (hydrocortisone acetate 3.75 mg.) 3 (hydrocortisone acetate 2.5 mg.) 4 (hydrocortisone acetate 1.25 mg) 5 days.

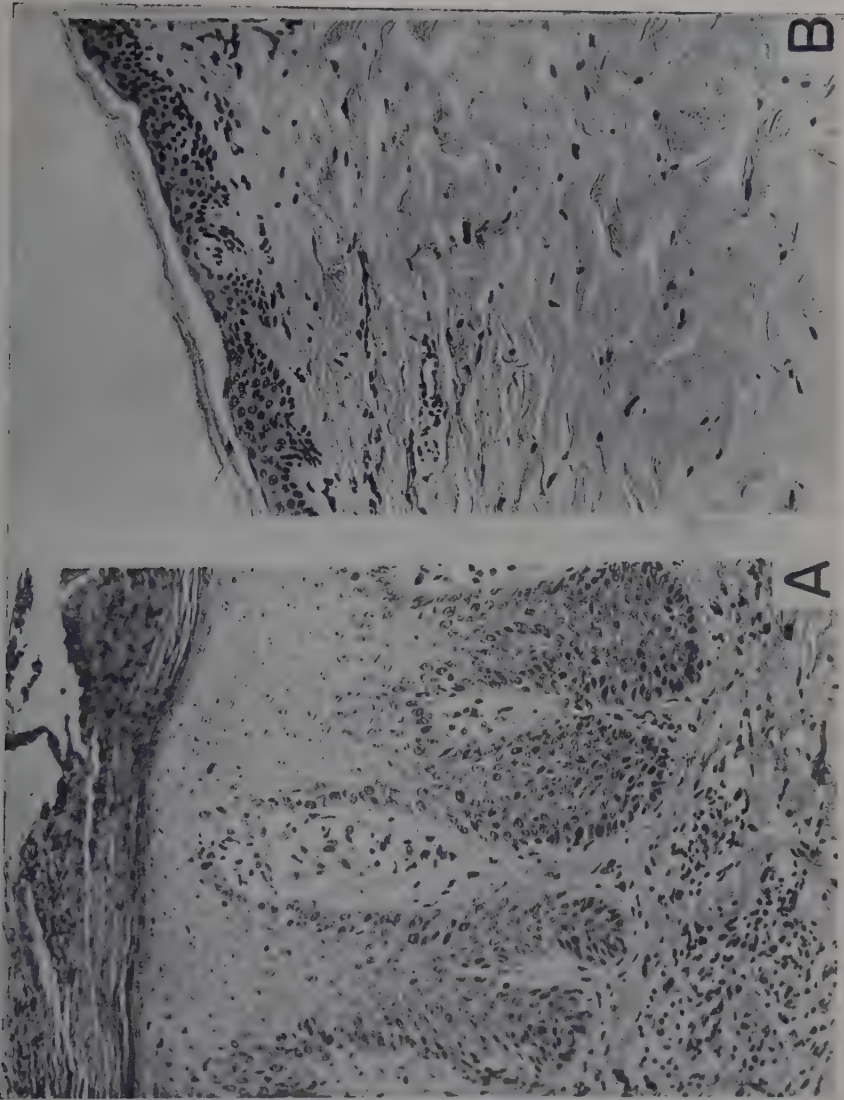


FIGURE 8. The psoriasis lesion after local injection of corticosteroids. (A) No change after injection of cortisone acetate—2 weeks. (B) After local injection of hydrocortisone acetate—2 weeks.  $\times 160$ , hematoxylin-eosin (from Goldman, O'Hara, and Basketof).



FIGURE 9. Showing technique of study of local activity of various corticosteroids in the tumor phase of mycosis fungoides inhibition 18 days after local injection 2.5 mg. hydrocortisone acetate.



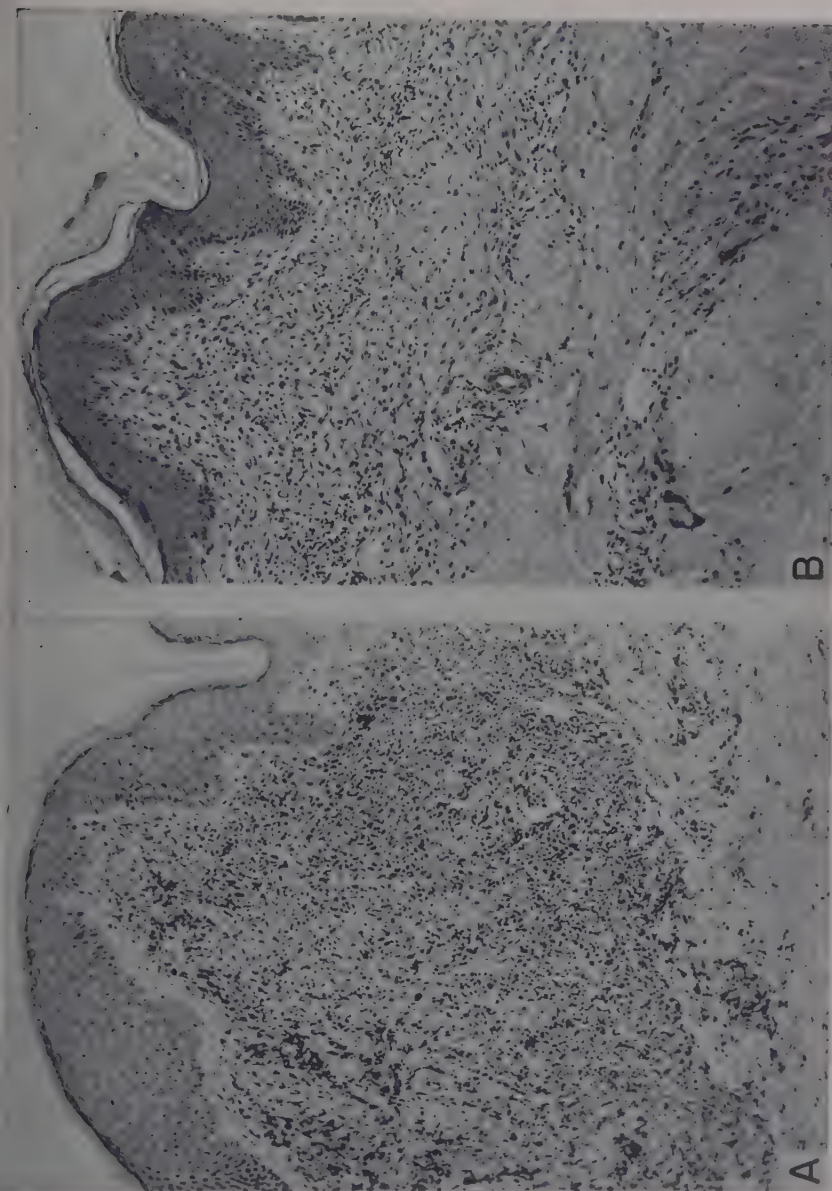


FIGURE 10. A. Tumor phase of mycosis fungoides.  $\times 100$ . B. Tumor phase of mycosis fungoides in the same patient just above an area injected with 2.5 mg. of hydrocortisone acetate 18 days.  $\times 160$  (hematoxylin-eosin).

The histopathology of the injection site has been studied in a variety of inflammatory and neoplastic reactions. No specific features of the inhibition of inflammation can be made out that could be related to the specific type of inflammatory reaction ("the nonspecific inhibition activity").

The study of histopathology in those conditions that are not affected by the local injection of the corticosteroid has also been of considerable interest. This type has included the urticarial lesion and, of course, also the histamine wheal and epidermal tumors, such as basal cell and squamous cell malignancies. The resistance of the urticarial lesion to the local action of hydrocortisone has been noted before.<sup>3</sup> In the epidermal tumors, there have been no recognizable changes in the invasive character of the tumors, although the inflammatory infiltrate has been decreased.

Detailed studies, after local application of both ointments and lotions of the hydrocortisone acetate and free alcohol and 9 $\alpha$  fluorohydrocortisone acetate and free alcohol, have shown no histopathologic reactions in normal skin. Chromatographic and colorimetric assay controls with hydrocortisone acetate and free alcohol also have revealed no evidence of absorption, in spite of definite local clinical responses. These studies of assay techniques are being done in bulla, in order to see if any more concentration can occur through easier absorption. It is obvious that our techniques do not pick up small amounts of topically applied materials, which are absorbed and which are active. Chromatographic studies are being investigated now after topical application of ointments and lotions of 9 $\alpha$  fluorohydrocortisone acetate and free alcohol to normal and pathologic skin.

Because of the interest<sup>6</sup> in the permeability of the blood vessel wall by leukotoxine in the study of local mechanisms of inhibition of inflammation, special attention has been directed in fixed sections of this vessel wall. In our sections, with our present techniques, except for increased perivascular collections, there is no evidence of structural change in the blood vessel wall. In the small blood vessel, immediately adjacent to the areas of maximum tissue response to the local deposition of the insoluble crystals, we are aware of the clinical picture of the changes in the late lesion, following the injection of corticosteroid, with its poikilodermatous appearance. The skin appendages adjacent to the hematoxylinophilic masses show no change.

It is apparent, then, that we still have to learn the mechanism of the changes that are produced locally by the injection of relatively large masses of the corticosteroid. We are also ignorant of the changes in the processes which make for the solution of these insoluble crystals into those agents which can affect inflammation. We are also unable to spread this depot of crystalline material over broad areas. Our techniques, however, are too crude at present to pick up the evidences of local suppression of inflammation by the topical application of very active materials applied to the surface of the skin. Our techniques also may not be enough to detect changes when only very small amounts of very active agents are injected locally.

*Summary*

Histopathological changes following the local injection of hydrocortisone and its analogs have been of interest in the study of the characteristic hematoxylinophilic masses produced locally in the skin of man. The local suppression of inflammation may be followed in some detail about the area of deposition of the hydrocortisones. These studies do not suggest any definite mechanism for the local suppression of inflammation. They do assist, however, in the evaluation of an assay technique for those materials which may have local activity in the skin of man. Our techniques at present are too crude to detect evidence of local changes in tissue produced by local application to the skin of the very effective local ointments and lotions.

*References*

1. GOLDMAN, L., R. FLATT & J. BASKETT. 1954. Technic of assay of an unknown steroid for possible local activity in the skin of man. *J. Investigative Dermatol.* **23**: 251.
2. GOLDMAN, L., H. O'HARA & J. BASKETT. 1952. The detection of crystals following local injection of cortisone and compound F into the skin of man. *Science*. **116**: 17.
3. GOLDMAN, L., H. O'HARA & J. BASKETT. 1953. A study of the local tissue reactions in man to cortisone and compound F. VI. Histopathological studies of the local effect of compound F in normal and pathological skin of man. *J. Investigative Dermatol.* **20**: 271.
4. ATKINSON, W. B., L. GOLDMAN, R. R. SUSKIND & J. BASKETT. 1954. The reaction of normal human skin to intradermal injection of hydrocortisone acetate. *J. Histochem. Cytochem.* **2**: 479.
5. OPDYKE, D. 1955. Personal communication to the author.
6. MENKIN, V. 1954. On the anti-inflammatory mechanism of hydrocortisone (compound F). *Science*. **120**: 1026.

# TOPICAL HYDROCORTISONE IN THE TREATMENT OF SKIN DISEASE

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It has now been almost 3 years since we first used hydrocortisone ointment in the treatment of selected diseases of the skin. Since our original report on 19 cases in 1952,<sup>1</sup> our additional experience in the management of many hundreds of patients and dozens of different dermatoses<sup>2-5</sup> makes it possible now to attempt to answer some of the questions which have been asked about the therapeutic use of hydrocortisone topically.

Altogether, we have studied over 40 preparations, including 6 chemical forms or derivatives of hydrocortisone, various concentrations of the steroids ranging from 0.1 per cent to 5 per cent, 14 different bases as vehicles for the steroids, and 9 combinations of the hydrocortisone compounds with various antibiotics.

The method of "symmetrical paired comparison"<sup>6</sup> was employed, whenever feasible, in order to evaluate simultaneously the therapeutic effectiveness of the various hydrocortisone compounds and bases which were used. This method consisted of using one preparation on one affected part of the body and comparing it with another preparation which was being used at the same time on symmetrically situated sites. In the beginning, we compared hydrocortisone acetate in a selected base with the base alone without hydrocortisone. As the studies progressed, different hydrocortisone compounds in the same base were compared one with another, different concentrations of the same hydrocortisone compounds were compared, the same hydrocortisone compounds in different bases were compared, and the various hydrocortisone preparations were compared with older established therapeutic measures.

This method of *progressive comparison* of new preparations with preparations we had already evaluated, permitted rapid and reasonably accurate conclusions concerning their relative therapeutic effectiveness, lack of primary irritancy, cosmetic acceptability, etc.

*For what dermatoses is the topical use of hydrocortisone indicated?* The following are among the dermatoses which have benefited by the topical application of hydrocortisone: (1) atopic dermatitis (including infantile eczema); (2) contact type eczematous dermatitis (including dermatitis of the hands); (3) anogenital pruritus; (4) dermatitis of eyelids, peribuccal or scrotal areas; (5) some forms of psoriasiform or seborrheic dermatitis, especially of the anogenital and intertriginous areas; (6) eczema of ear canals (otitis externa); (7) nummular eczema; (8) eczema of nipples and areolae; (9) exudative discoid and lichenoid chronic dermatosis (Sulzberger and Garbe); and (10) miscellaneous lichenified eczemas and circumscribed lichen simplex.

*For what dermatoses has topical hydrocortisone not proven beneficial?* The following dermatoses, among others, are not benefited by the topical applica-



tion of hydrocortisone: (1) dermatitis herpetiformis; (2) pemphigus; (3) alopecia areata; (4) chronic discoid lupus erythematosus; (5) drug eruptions (*e.g.* erythema multiforme-like, purpuric); (6) photo-sensitivity eruptions; (7) *verruca vulgaris*; (8) generalized pruritus; and (9) psoriasis (*e.g.* generalized, guttate, and plaque forms).

*Are there any contraindications to the topical use of hydrocortisone?* The topical use of cortisone or hydrocortisone is contraindicated for herpes simplex infections of the eye (dendritic ulcer).<sup>7</sup> Our clinical experience suggests that this may be the case also in herpes simplex of the skin and mucous membranes sometimes retarding healing, or perhaps, even aggravating the existing lesions. Moreover, recent experimental work of N. Kanof, at the New York Skin and Cancer Unit, indicates that second degree thermal burns are often made worse by topical applications of hydrocortisone. With these exceptions, there are, as far as I know, no contraindications to the external use of hydrocortisone in dermatologic therapy.

Contrary to the contraindications and occurrence of undesirable side effects that may be expected from cortisone and hydrocortisone internally, such is not the case for topical applications of hydrocortisone. Whereas there is always the fear of lighting up some latent infection with oral cortisone or hydrocortisone, this has not been proved to occur when hydrocortisone is applied locally. In our experience, the incidence of spread of superficial infections following the use of topical hydrocortisone is no greater than with any other ointment not having antibacterial or antiseptic properties. It is of interest that topical hydrocortisone has not been shown to produce acneform eruptions, hypertrichosis, sodium retention and edema, hemorrhages, or to retard wound healing. This is in contrast to large oral or parenteral doses of cortisone or hydrocortisone which are known to be responsible for some or all of these effects. While the mechanisms of action of cortisone and hydrocortisone internally, and of hydrocortisone externally, in benefiting certain dermatoses is not known, various mechanisms have been postulated. Certainly there is insufficient evidence to date to make one believe that hydrocortisone, applied topically, works in any way other than by its local effect; that is, that its beneficial effects are not produced by way of absorption and "systemic" action. There is no question but that the mechanism of action of these compounds needs to be studied further.

*What hydrocortisone compounds are available for topical use?* Hydrocortisone acetate and hydrocortisone (free alcohol) are now commercially available in various vehicles for topical application. Both of these compounds are therapeutically effective and, often, equally so. There are, however, some cases in which either one or the other of these two preparations proves superior and, in these instances, the free alcohol is the better more often than the acetate. This is certainly true for the 1 per cent and 2.5 per cent concentrations. The most recent hydrocortisone derivative to be made available for topical use is 9- $\alpha$ -fluorohydrocortisone acetate. This new material will be discussed later in this monograph by Doctor Marion B. Sulzberger.

*What are the optimum concentrations of these compounds for topical use?* In general, it may be stated that the 2.5 per cent concentration of the hydro-

cortisone acetate or free alcohol is more effective than the 1 per cent, and that the 1 per cent concentration is more effective than the 0.5 per cent. We have not been impressed with the effectiveness of the acetate in concentrations less than 1 per cent. Only recently have we observed that 0.1 per cent, 0.2 per cent and 0.5 per cent concentrations of the free alcohol were reasonably effective in some instances. This observation requires further investigation, however, before any definite conclusions can be drawn.

In order to judge the effectiveness of a particular topical hydrocortisone preparation in the treatment of a skin disease, it seems logical to begin treatment with the strongest concentration. If the strongest concentration proves effective, then preparations of lesser concentrations may be tried.

*Is the therapeutic effectiveness of topical hydrocortisone affected by the vehicle?* It has not been our impression that the therapeutic effectiveness of topical hydrocortisone is influenced very much one way or the other by the particular vehicle in which it is incorporated. Newer bases are being introduced, however, and it is possible that some differences will be noted in the future.

All of the ointment and lotion vehicles commercially available today generally are well tolerated. There are, however, certain noticeable exceptions in which one base is better tolerated or more effective than another. On occasion, the carbowax type bases are somewhat irritating. The plain petrolatum and lanolin-petrolatum bases are, in general, more lubricating and are usually well tolerated and often preferred around the eyelids, on the lips, on the anogenital areas, and on dry fissured parts. They have proved to be excellent bases for hydrocortisone in the treatment of infantile eczema. Of course, the lanolin-containing bases cannot be used on the small minority of individuals known to possess allergic sensitivity to lanolin. In general, the lotions are pleasant to use, spread easily, having large coverage, are cosmetically acceptable, and are often preferred by the patient.

*How soon after starting therapy with topical hydrocortisone are the beneficial effects noted?* When improvement follows the use of topical hydrocortisone preparations, it is usually noted within 24 to 48 hours after starting treatment. Patients often volunteered the information that their itching, burning, stinging, and other discomfort was relieved very rapidly. Objective improvement was also evident in many cases within the first day or two after starting treatment. Only rarely were beneficial effects observed after an initial lag period of several days to a week or two, during which time the hydrocortisone was apparently ineffective.

*Are the initial benefits derived from topical hydrocortisone preparations maintained with their continued use?* It has been our experience that the beneficial effects derived from the topical use of hydrocortisone are usually maintained with continued application of the medications. It is sometimes necessary, in the face of flare-ups, to use stronger preparations or to increase the frequency of the applications. Only infrequently have the drugs lost their initial effectiveness.

Whereas, in the beginning of treatment, three applications a day are often necessary, with continued use the frequency of inunction may be reduced,

sometimes considerably. In some dermatoses which do not ordinarily undergo spontaneous involution, as little as one application a week has proved sufficient to maintain the beneficial effects once they have been achieved; for example, pruritus ani and/or vulvae. One application every day or every other day will sometimes control atopic dermatitis. While topical hydrocortisone will completely relieve the signs and symptoms in some cases, there are many instances in which there is a limit to the degree of beneficial response that may be achieved with these preparations; *i.e.*, there is a point at which the improvement, both subjectively and objectively, can not be increased either by the use of available stronger preparations or by more frequent applications.

*Are the beneficial effects derived from topical hydrocortisone preparations maintained when their use is discontinued?* As would be expected in the treatment of self-limited dermatoses, the beneficial effects of topically applied hydrocortisone are maintained when their use is discontinued. Our experience in the treatment of those dermatoses which ordinarily do not undergo "spontaneous" involution has already been mentioned. In a few instances, we have been of the opinion that, following the long-term continued application of topical hydrocortisone, not only can the frequency of inunction be reduced, but it can sometimes be *discontinued* altogether without recurrence of the disease. This has been particularly true for a few cases of chronic hand eczemas, pruritus ani and/or vulvae, and atopic dermatitis. While these instances have been few, the fact of their occurrence is encouraging. It is interesting to note that, in this respect, the response to topical hydrocortisone is not unlike the gradual reduction of oral cortisone dose and its eventual discontinuation without recurrence of the dermatoses under treatment, as previously reported.<sup>8</sup>

While the possibility of unrelated or "spontaneous" remissions of these dermatoses should be considered, it is not likely that such was the case in every instance in which a remission was obtained and then persisted without further topical applications of the hormone.

*Will allergic sensitization or adverse systemic effects occur with the long-term use of these compounds?* Thus far, to our knowledge, there have been no instances of an allergic contact dermatitis resulting from the topical use of hydrocortisone. Nor have there been any reports or evidence that undesirable systemic effects are produced by the repeated and long-term use of these compounds even over extensive body areas.<sup>9-12</sup> Doctor Conrad Smith has reported, elsewhere in this monograph, on the various laboratory evidences which speak against the absorption of hydrocortisone ointments through apparently normal and diseased skin.<sup>13, 14</sup> These findings are entirely in keeping with our clinical observations.

In spite of the many favorable properties of these medications, they should not be relied upon to do the job alone in every instance.

The use of topical hydrocortisone alternately with older established remedies is of benefit in certain cases. Where irritation follows the use of chrysarobin or Anthralin in the treatment of psoriasis; where excessive dryness and irritation follows the use of sulfur and resorcin in the treatment of acne, particularly around the eye lids, mouth and on the neck; and where various other medications temporarily irritate such areas as the axillae, groins, penis, scrotum, *etc.*,



hydrocortisone in ointment or lotion form is often beneficial in producing rapid soothing so that the more effective remedy may be used once again.

In addition, there are dermatoses in which the combined use of hydrocortisone and tar, or of hydrocortisone and chrysarobin are more beneficial than the use of either drug alone. The beneficial effects that may be derived from various combinations of hydrocortisone and other topical medications provide a fertile field for future investigation.

Search for possible causative factors of the dermatitis under treatment, of course, should be carried out diligently in all cases. Wherever possible, these factors should be discovered and eliminated.

Hydrocortisone locally also has proved of value in combination with oral and other systemic therapy. A good example of this are cases in which cortisone orally is necessary to control a severe dermatitis and where the concomitant topical application of hydrocortisone will sometimes make it possible to reduce the oral dose to a level where the undesirable side effects are no longer a matter of concern. In some instances, the dose of the oral steroid may be reduced gradually and even discontinued while the topical preparation is being used regularly.

### *Conclusions*

According to the present evidence, hydrocortisone preparations promise to be among the most important additions ever made to the list of topical medications that may be used in the treatment of diseases of the skin. In this respect, they may be considered to be almost ideal therapeutic agents, in that they are: (1) rapidly effective in a great many cases of common dermatoses; (2) effective in small amounts; (3) of sustained effectiveness in most cases; (4) easily applied, cosmetically acceptable, and easily removed; (5) not prone to produce allergic sensitization; (6) not prone to act as primary irritants; and (7) not absorbed to produce undesirable systemic effects.

### *References*

1. SULZBERGER, M. B. & V. H. WITTEN. 1952. The effect of topically applied compound F in selected dermatoses. *J. Investigative Dermatol.* **19**: 101.
2. SULZBERGER, M. B., V. H. WITTEN & C. C. SMITH. 1953. Hydrocortisone (compound F) acetate ointment in dermatological therapy. *J. Am. Med. Assoc.* **151**: 468.
3. SULZBERGER, M. B., V. H. WITTEN, & C. C. SMITH. 1953. Hydrocortisone (compound F) free alcohol ointment. Correspondence. *J. Am. Med. Assoc.* **152**: 1456.
4. WITTEN, V. H., A. A. AMLER, M. B. SULZBERGER & A. G. DE SANCTIS. 1954. Hydrocortisone ointment in the treatment of infantile eczema. *Am. J. Diseases Children.* **87**: 298.
5. SULZBERGER, M. B. & V. H. WITTEN. 1954. Hydrocortisone ointment in dermatological therapy. *Med. Clinics N. Amer.* **38**: 321.
6. SULZBERGER, M. B., R. L. BAER, A. KANOF & C. LOWENBERG. 1946. Methods for the rapid evaluation of the beneficial and harmful effects of agents applied to the human skin. *J. Investigative Dermatol.* **7**: 227.
7. GORDON, D. M. 1954. The Clinical Use of Corticotropin, Cortisone and Hydrocortisone in Eye Disease. Thomas. Springfield, Ill.
8. SULZBERGER, M. B. & V. H. WITTEN. 1954. Prolonged therapy with cortisone for chronic skin diseases. *J. Am. Med. Assoc.* **155**: 954.
9. SIDI, E., J. BOURGEOIS-GAVARDIN & G. PLAS. 1953. Topical application of hydrocortisone acetate in treatment of eczema and pruritus. *Presse méd.* **61**: 992.



10. ROBINSON, H. M. & R. C. V. ROBINSON. 1954. Treatment of dermatoses with local application of hydrocortisone acetate. *J. Am. Med. Assoc.* **155**: 1213.
11. MALKINSON, F. D. & G. C. WELLS. 1954. Clinical experience with hydrocortisone ointment. *Brit. J. Dermatol.* **66**: 300.
12. MCCORRISTON, L. R. 1954. Hydrocortisone (compound F) acetate ointment in eczemas of infants and children. *Can. Med. Assoc. J.* **70**: 59.
13. SMITH, C. C. 1953. The eosinophilic response after inunction of hydrocortisone ointment; experiment demonstrating lack of significant absorption and of systemic effects. *Arch. Dermatol. & Syphilol.* **68**: 30.
14. WITTEN, V. H., A. J. SHAPIRO & R. M. SILBER. 1955. Attempts to demonstrate absorption of hydrocortisone by new chemical test following inunction into human skin. *Proc. Soc. Exptl. Biol. Med.* In press.

## TOPICAL HYDROCORTISONE AND NEOMYCIN IN THE EXTERNAL EAR\*

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In this era of new and remarkable chemotherapeutic agents and antibiotics, it is not unusual to find reports of excellent results in the treatment of recalcitrant otic infections. There have been few topical medications introduced in recent years, however, which have proved of lasting value in the treatment of skin irritations in and around the external ear. Hydrocortisone acetate (compound F) alone and combined with certain antibiotics in proper vehicles seems to offer a solution to some of these difficult otologic problems.

The most common, and frequently the most resistant, dermatoses found in the otologist's office include simple pruritus of the ear canal, localized neurodermatitis of the auricle, periauricular regions and the meatus of the ear canal, diffuse external otitis, and postoperative infections of the skin lining the mastoid and fenestration cavities. These dermatoses have been treated with some success by topical antibiotics, chemotherapeutic agents, and radiation. There has been, however, a high incidence of chemical dermatitis and fungus disease superimposed upon the original lesion in the mastoid and fenestration cavities as a result of the insufflation of antibiotic and chemotherapeutic preparations. Radiation therapy has offered excellent temporary improvement in exposed skin areas, but has no value in the external auditory canal or mastoid cavity. It has been misused in many localities and its frequent application is fraught with danger.

*Literature review.* There have been many reports describing excellent results from the use of hydrocortisone acetate (compound F)<sup>1-5</sup> and a combination of hydrocortisone and neomycin for various dermatoses.<sup>6</sup> The latter has been shown to have a marked bacteriostatic and bactericidal effect upon a wide range of bacteria<sup>7</sup> including a variety of Gram-negative and Gram-positive organisms. It appears to be active against certain strains of *Escherichia coli* (*E. coli*) and *proteus*.<sup>8-10</sup> Of particular importance is the low index of sensitivity described in various studies. One investigator<sup>11</sup> reported localized cutaneous moniliasis following the application of neomycin, and another<sup>12</sup> described a contact type of sensitization which apparently occurred five months or more after treatment began.

Of particular importance to the otologist is the report by Sulzberger and Rein,<sup>13</sup> showing that hydrocortisone ointment, topically applied to the ear, was effective in relieving inflammation and itching in cases of external otitis, and that by Baer and Litt<sup>14</sup> describing the use of a hydrocortisone, neomycin suspension in 10 cases of external otitis. More recent reports have amplified these studies.<sup>15, 16</sup>

*Methods and procedures.* The greatest problem that we encounter in teach-

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ing proper therapy is the failure of the otolaryngologist to appreciate the large number and variety of dermatoses which are encountered in and around the external ear. Because, occasionally, there arises some question as to the dermatologic entities about which the otologist writes, I shall define the various dermatoses included in this presentation,<sup>17</sup> alluding briefly to the histology of the skin of this area. This procedure I believe, will give us a better understanding of the problems encountered in the use of hydrocortisone in the external ear.

The skin of the auricle is provided with a distinct subcutaneous layer on its posterior convex surface only. It carries a few small hairs with sebaceous glands of considerable size. These are particularly evident in the concha. The sweat glands are of the eccrine type, and the largest concentration of these glands is found in the preauricular region and in the postauricular skin along the hair line and down onto the mastoid process and neck.<sup>18</sup>

The auricle and external auditory canal show great individual variations in size and shape. In some cases, the tragus and antitragus form a marked obstruction at the entrance of the ear canal, while the long axes of the two canals form an obtuse angle. Some lumina are wide and straight without any constriction and thus allow easy access to fluids, while others are narrow and present curvatures which serve to make the entrance or removal of fluids difficult.

The external auditory canal is composed of (1) a cartilaginous canal, and (2) a bony canal. The cartilaginous canal is pliable and flexible as a result of the structure of the cartilage and the vertical fissures in the anterior wall. The narrowest part of the ear canal, the isthmus, is formed by the marked convexity of the floor and anterior wall of the osseous portion. The canal widens abruptly at the drum to form the sulcus of the tympanic membrane.

The skin lining the cartilaginous portion of the external auditory canal is thicker than that lining the osseous portion, shows very little subcutaneous tissue, and is firmly attached to the perichondrium. There is a fairly well-marked layer of stratum corneum, and definite papillae may be visualized. No eccrine glands can be observed in histological preparations<sup>19, 20</sup> or in sweating experiments.<sup>21, 18</sup> The sebaceous glands attached to the hair follicles are exceptionally large, and most of the apocrine glands may be seen draining into the apo-pilo-sebaceous units.<sup>22, 23</sup> In the osseous canal, the skin is thin and adheres to the periosteum. There is an absence of papillae, little dermis, and no subcutaneous tissue. Apocrine and sebaceous glands are rarely found.

Let us take a moment to consider the histology of these epidermal glands. The sebaceous glands lie superficially in the dermis and consist of multilobulated grapelike glands, all of them opening through short excretory ducts into the lumen of the hair follicle (follicular canal). The excretory ducts are lined by stratified squamous epithelium, which is continuous with the external root sheath of the hair follicle and with the Malpighian layer of the epidermis. Most of the polyhedral cells making up the alveoli of the sebaceous glands are filled with fat droplets. As one progresses towards the duct, the nuclei are seen to shrink and then disappear as the cells break down into the fatty detritus

which forms the sebum and which ultimately mixes with the apocrine secretion and the horny exfoliated proteinlike scales in the follicular canals.

There is no direct evidence for a secretory innervation of the sebaceous glands at the present time. It has been suggested on the basis of morphological data that the sebaceous glands are supplied by nerves which form a plexus on the basement membrane of the glands. But there is no proof that these unmyelinated fibers actually enter the parenchyma or that they stimulate glandular activity. It has been proposed that emotional tensions increase sebaceous gland activity by way of direct nervous stimulation.<sup>23</sup>

The apocrine glands are located most abundantly in the skin of the second and third quarters of the external auditory canal and, according to Von Troltsch,<sup>24</sup> extend along the superior wall almost to the tympanic membrane. Alzheimer<sup>25</sup> reported a great concentration of apocrine glands both on the superior and inferior walls. The apocrine glands develop as outgrowths of the outer sheath of the hair follicles (in contrast to the eccrine sweat glands which develop as direct downward projections from the epidermis). The highest percentage of the apocrine excretory ducts open into the follicular canal while a small percentage of these glands open independently upon the surface of the skin of the ear canal.<sup>21, 22</sup>

As shown by Shelley and Hurley in observations on axillary apocrine glands,<sup>26</sup> apocrine sweat is a milky whitish or grayish, sometimes yellowish secretion, which forms a light-colored cone over the poral orifice. It takes the form of an adherent cap which spreads over the perifollicular skin in a thin film. Autonomic innervation is easily demonstrable. After painful stimulation, the local injection of epinephrine, or intense emotional reactions, a droplet may appear after a latent period of 15 seconds or more. Once a droplet has been secreted, apocrine function ceases for several hours, and further stimulation is ineffective. Apocrine glands do not appear to respond to thermal stimulation.

It appears that these glands may have an important role in washing the more viscid sebum from the hair follicle and mixing with it to form the adherent protective surface coating. It is worth emphasizing, as presented by Rothman,<sup>23</sup> that the stratum corneum offers little or no barrier to infection since it is perforated by the apo-pilo-sebaceous units and thus allows ready contact between external agents and living cells of the sebaceous and apocrine glands. The waxy secretion, when present on the surface and within the follicular canal, prevents the permeation of noxious agents through the epidermis.

#### TYPES OF EXTERNAL OTITIS

##### (1) *Neurogenic*

(a) *Simple pruritus*. This is predominantly an affliction of busy executives and middle-aged women. Such persons are tense, hyperactive, and very voluble about their sufferings. There is usually intense itching. They tug and scratch their ears and use any available instrument to relieve the persistent itching sensation. Examination reveals an erythematous, scaly lesion limited to the external meatus of the auditory canal, while the auricle and the medial four fifths of the canal are free of any involvement. Although cultures



show a predominance of Gram-positive organisms, occasionally Gram-negative bacilli are seen.

(b) *Localized neurodermatitis*. These cases are characterized by various-sized circumscribed, elevated, excoriated, scaly patches in the concha and in the outer one fourth of the external auditory canal. Other isolated plaques may be found, occasionally, on the lobule of the auricle, the eyelids, the sides and back of the neck, and the antecubital areas. Cultures show primarily Gram-positive organisms.

### (2) *Diffuse External Otitis*

This is an acute or chronic symptom complex present mainly during hot, humid weather. The mild acute case has slight pain on manipulation of the auricle. The skin of the ear canal reveals some edema, slight redness, and a coating of odorless, adherent secretion or exfoliated debris. In the more severe acute cases, patients complain of intense pain on mastication and on manipulation of the external ear. There is marked periauricular edema and partial or complete obliteration of the canal lumen by the edematous walls. Gray or green seropurulent secretions and sheets of exfoliated debris are seen in the remaining lumen. The skin of the canal is thickened, purplish red in color, and may have on the superior and inferior walls a papular appearance resembling gooseflesh.

In the chronic case, a diffuse thickening of the skin of the entire canal is found. There may be a fetid gray-brown or greenish secretion coating the skin and filling the tympanic recess. Papules and vesicles are sometimes seen, and the drum is thickened and lusterless.

Cultures of the acute and chronic cases almost invariably show an abundant growth of Gram-negative bacilli although occasionally fungi are found. Stained smears reveal myriads of bacilli and epithelial cells.

### (3) *Dermatitis of the Skin Lining the Mastoid and Fenestration Cavities*

In a small number of cases, drainage persists from the post-operative mastoid or fenestration cavities despite active therapeutic measures. Examination reveals a greenish-gray exudate coating the cavity. The skin lining is thickened, reddened, and ulcerated. Various sized granular islands are present, especially on the superior wall of the mastoid cavity. Cultures usually show a mixture of Gram-positive organisms and Gram-negative bacilli although fungi may be cultured.

### (4) *Otomycosis*

Diseases falling under this heading are chronic or recurrent. The patient complains of intense itching, a feeling of fullness in the ear, difficulty in hearing and, later, severe pain. On examination, one may find moist cerumen intermixed with exfoliated scales and sheets of epithelium, or the depths of the canal may be filled with wet grayish "blotting paper" debris. In uncomplicated cases, the organisms are seen as green, black, or white filamentous structures. Direct wet unstained smears reveal the presence of mycelia, spores,

neutrophiles, and myriads of epithelial cells. The so-called saprophytic fungi make up the largest number of these organisms and include strains of *Aspergilli Mucor*, *Monilia* and *Penicillium*.

In this study, the following categories of diseases and pharmaceutical preparations were evaluated.

*Category A.* A neomycin-hydrocortisone acetate ointment (1.5 per cent)\* alone was applied to 21 ears showing neurogenic lesions in the concha, at the external meatus, or in and around the auricle. This ointment, in conjunction with hydrocortisone-neomycin drops,† was used in 29 ears which showed similar lesions. The patients were instructed to remove the adherent crusts or scales with mineral oil or vaseline and then to apply the ointment three times a day. If weeping and vesiculation were present, mild aluminum acetate packs were used as a cleansing astringent prior to application of the ointment.

*Category B.* Neomycin-hydrocortisone acetate drops (1.5 per cent) in an aqueous vehicle‡ were used in postfenestration and postmastoid cavities, in patients with otitis media, diffuse external otitis, and undiagnosed otomycosis (82 ears). Prior to the institution of therapy, the cavity was cleansed by irrigating with 3 per cent hypertonic saline, and all sheets of exfoliated debris were removed with forceps or suction. When present, granulations were removed with a curette and, when otomycosis was diagnosed, treatment with proper fungicidal agents was instituted. The patient was instructed to lie on the unaffected side, instill five drops of the suspension into the involved external ear three times a day for five minutes. In occasional cases, the ointment was applied to a wad of cotton which was then packed in the mastoid cavity and allowed to remain for 24 hours.

Irrigations were repeated as often as necessary on follow-up visits in order to keep the cavity clean and permit the therapeutic agent to come in close contact with the irritated or infected skin. Cultures and smears were obtained both pretreatment and posttreatment as often as possible, and the bacteriologic changes were recorded.

*Category C.* In 57 ears, hydrocortisone ointment‡ or neomycin hydrocortisone ointment§ was applied on nylon strips and inserted into the clean cavity at the time of fenestration or mastoid surgery. This was allowed to remain in place for approximately five days and was removed at the time of the first dressing. During the course of follow-up care, hydrocortisone ointment, neomycin-hydrocortisone ointment or neomycin, hydrocortisone drops were applied. Topical hydrocortisone preparations were used only during the postoperative course in another 81 ear cavities.

The pertinent data and findings of the 90 treated patients (114 ears) included in categories A and B are summarized in TABLES 1 and 2.

Our practice includes a high percentage of young adults and children, and we see an equal number of males and females. Therefore, it is noteworthy that

\* Neo-Cortef ointment (Upjohn Company, Kalamazoo, Mich.) containing 15 mg. of hydrocortisone acetate (compound F) and 5 mg. of neomycin sulfate per gram of bland ointment.

† Suspension of neo-Cortef acetate (1.5 per cent) containing 15 mg. of hydrocortisone acetate (compound F) and 5 mg. of neomycin sulfate per cc. of an aqueous vehicle (Upjohn Company).

‡ Cortef, (1 per cent), Upjohn Company, and Hydrocortone Acetate 1 per cent Topical Ointment (hydrocortisone acetate), Merck Co., Inc., Rahway, N. J.

§ Neo-Cortef ointment (1.5 per cent) Upjohn Company.

TABLE 1

SHOWING DATA PERTINENT TO THE TREATMENT OF VARIOUS OTIC DISEASES WITH PREPARATIONS OF NEOMYCIN AND HYDROCORTISONE ACETATE

Category	Average age	No. pts.	No. ears	Ear involved		Sex		Form of medication		
				Rt.	Left	F	M	O	D	O & D
	yr.									
N.E.O.....	40	22	32	16	16	22	10	21	4	7
A.D.E.O.....	35	22	28	15	13	17	11	7	10	11
C.D.E.O.....	48	6	10	5	5	5	5	1	4	5
O.M.....	40	16	18	11	7	8	10	0	17	1
P.O.M.....	24	9	9	2	7	6	3	0	8	1
P.O.F.....	44	4	4	2	2	3	1	0	3	1
Otomycosis.....	26	8	9	5	4	5	4	2	7	0
Miscellaneous.....	20	3	4	1	3	1	3	1	0	3
Totals.....	35	90	114	57	57	67	47	32	53	29

N.E.O.—Neurogenic external otitis; A.D.E.O.—Acute diffuse external otitis; C.D.E.O.—Chronic diffuse external otitis; O.M.—Otitis media; P.O.M.—Postoperative infection of mastoid cavity; P.O.F.—Postoperative infection of fenestration cavity; Misc.—Perichondritis, infantile dermatitis, etc.; O—Ointment of neomycin-hydrocortisone acetate; D—Drops of suspension of neomycin-hydrocortisone acetate.

TABLE 2

SHOWING DATA PERTINENT TO THE TREATMENT OF VARIOUS OTIC DISEASES WITH PREPARATIONS OF NEOMYCIN AND HYDROCORTISONE ACETATE

Category*	No. pts.	No. ears	Pre-dominant Gram-positive	Bacteria Gram-negative	No growth	No culture	Exc.	Response		Poor
								Good	Fair	
N.E.O.....	22	32	10	9	3	10	14	16	1	1
A.D.E.O.....	22	28	4	16	1	7	11	12	2	3
C.D.E.O.....	6	10	1	9	0	0	3	5	0	2
O.M.....	16	18	9	6	1	2	10	5	0	3
P.O.M.....	9	9	6	1	2	0	3	4	1	1
P.O.F.....	4	4	2	1	0	1	2	0	1	1
Otomycosis.....	8	9	2	2	1	4	0	3	1	5
Miscellaneous.....	3	4	3	1	0	0	0	3	0	1
Totals.....	90	114	37	45	8	24	43	48	6	17

\* See TABLE 1 for key.

there is a predominance of females in the neurogenic and acute diffuse groups. The average age of all groups is older than one would anticipate. There is no predilection for one or the other ear.

It is significant that Gram-negative organisms, primarily *E. coli* and *Pseudomonas*, were cultured in more than half of the so-called neurogenic ears. Few streptococci were present in our cultures although actively sought. It was not unexpected that almost all the cultures of the diffuse external otitis group showed a predominant growth of Gram-negative bacilli.

The most significant clinical observation is the consistent and excellent response obtained in that group of indolent and recalcitrant ears placed in the neurogenic category. Only in occasional cases were indifferent results observed. There was prompt control of the itching, crusting, scaling, and weeping. Mild exacerbations were treated easily by the patient. Thus the neces-

sity for radiotherapy was eliminated in every case in this series. Needless to say, exacerbations occurred in those cases where psychosomatic factors contributed to the production of the lesion. In these cases, an effort was made to acquaint the patient with the underlying psychogenic basis for the dermatosis.

It was not anticipated that hydrocortisone would be effective in the treatment of acute diffuse external otitis, in view of the previously described pathologic changes and superimposed infection with Gram-negative bacilli. When neomycin was combined with hydrocortisone, however, the response was found to be good in the uncomplicated cases.

Of special importance to the otologist is the response obtained in the treatment of the chronically irritated skin lining the mastoid and fenestration cavities. These cavities which had been draining for years and showed ulceration and granulation, and from which strains of *Pseudomonas* and *Proteus* were cultured, responded promptly to the application of hydrocortisone-neomycin drops. The skin-lining rapidly assumed a healthy color, suppuration subsided, and islands of granulation disappeared, producing an intact, dry, skin-lined cavity.

Fungi were reported in a surprisingly high percentage of cases of chronically draining postoperative mastoid and fenestration cavities. It is therefore not unexpected that, prior to the receipt of the culture report, fungi flourished and some of these cases did poorly while treated with neomycin and hydrocortisone. Two patients included in Group B, treated for persistent postmastoidectomy otorrhea, developed otomycosis which was superimposed on the original disease. Fungus infections occurred in one patient with chronic otitis media and in another with postfenestration otorrhea. It was shown that latent otomycosis antedated the use of the neomycin-hydrocortisone in these latter two cases. Thus, there were 4 cases of otomycosis in 114 ears included in categories A and B.

A striking contrast was found among the patients in category C. In the 57 ears where the ointment was packed into or applied to the mastoid or fenestration cavity, fungus infection appeared in 32 per cent of the ears some time during the postoperative course. In the 81 similar cases in which various antibiotic and chemotherapeutic preparations were applied to the mastoid cavity only during the postoperative period, a fungus incidence of 28 per cent was found.

In all cases where cultures of fungi were obtained, various strains of *Aspergilli* were isolated,\* including the niger, terrius, and glaucus group variety. Treatment with fungicides and gentian violet in alcohol resulted in eradication of the fungi.

The tendency for *Aspergilli* to grow luxuriantly on the skin lining the mastoid or fenestration cavity following the application of various antibiotics during postoperative observation is as yet unexplained. This was previously noted following the use of topical Aureomycin and terramycin in the ear<sup>21</sup> and is now being observed following the use of neomycin-hydrocortisone preparations.

In one instance, sensitivity to neomycin-hydrocortisone was observed and

\* We are indebted to Doctor Morris Moore for mycological consultation in these cases.



subsided promptly after the discontinuance of the drops. In a superficial review of preoperative and postoperative pure tone audiograms and speech tests of 138 fenestration patients who received neomycin-hydrocortisone preparations, no hearing loss was observed which could be attributed to the chemotherapeutic preparations.

In general, this study points up the increasing necessity for the clinician to understand clearly the differential diagnosis of diseases of the external ear. Only by appreciating the morphologic characteristics and the associated bacterial flora usually present in the varied and distinct categories of diseases of the external ear can prompt and satisfactory therapy be applied. If we are to avoid exacerbations of disease, superimposed otomycosis and sensitization following the use of hydrocortisone, more attention must be given to the proper recognition of these various categories.

Of interest is the striking therapeutic results obtained with the aqueous preparation of hydrocortisone in the inaccessible areas of the ear. Further consideration should be given to proper vehicles as carriers of these potent steroids and effective combinations of antibiotics, fungistatic agents, and steroids for the treatment of diseases of the external and middle ear.

Plans have been made for a study of the unexplained environmental conditions and factors which contribute to an increased incidence of otomycosis in postoperative mastoid cavities.

### References

1. SULZBERGER, M. B. & V. H. WITTEN. 1952. The effect of topically applied compound F in selected dermatoses. *J. Investigative Dermatol.* **19**: 101-102.
2. SULZBERGER, M. B., V. H. WITTEN & C. C. SMITH. 1953. Hydrocortisone compound F free alcohol ointment. *J. Am. Med. Assoc.* 1456.
3. WITTEN, V. H., A. B. AMLER, M. B. SULZBERGER & A. G. DESANCTIS. 1954. Hydrocortisone ointment in the treatment of infantile eczema. *Am. J. Diseases Children.* **87**(3): 298-304.
4. MCCORRISTON, L. R. 1954. Hydrocortisone compound F acetate ointment in eczema of infants and children. *Can. Med. Assoc. J.* **70**: 59-62.
5. ROBINSON, R. V. 1953. Local use of hydrocortisone acetate: a preliminary report. *Bull. Johns Hopkins Hosp.* **93**(3): 147-149.
6. GOLDMAN, L., H. O'HARA & J. BASKETT. 1953. A study of the local tissue reactions in man to cortisone and compound F. *J. Investigative Dermatol.* **20**: 271-283.
7. WAKSMAN, S. A., E. KATZ & H. LECHEVALIER. 1950. Antimicrobial properties of neomycin. *J. Lab. Clin. Med.* **36**: 93-99.
8. WEISS, D. & S. A. WAKSMAN. 1950. Strain specificity and production of antibiotic substances. *Proc. Natl. Acad. Sci. U. S.* **36**(5): 293-300.
9. WAISBREN, B. A. & W. W. SPINK. 1950. A clinical appraisal of neomycin. *Ann. Internal Med.* **33**(5): 1099-1119.
10. WARTH, T., C. A. CHANDLER & E. A. BLISS. 1950. The antibacterial action of neomycin and furadroxyl *in vitro* and *in vivo*. *Bull. Johns Hopkins Hosp.* **86**: 169-178.
11. LIVINGOOD, C. S., S. NILASENA, W. C. KING, R. A. STEVENSON & J. F. MULLENS. 1952. Pyogenic infections treated by neomycin. *J. Am. Med. Assoc.* **148**: 334-339.
12. BAER, R. L. & J. S. LUDWIG. 1952. Allergic eczematous sensitization to neomycin. *Ann. Allergy.* **10**: 136-137.
13. SULZBERGER, M. B. & C. R. REIN. 1953. The present status of hydrocortisone acetate ointment in dermatology therapy. *Arch. Dermatol. and Syphilol.* **68**: 451.
14. BAER, R. L. & J. Z. LITT. 1954. Treatment of otitis externa with hydrocortisone suspension. *J. Am. Med. Assoc.* **155**(11): 973.
15. SENTURIA, B. H. & V. ALFORD. 1954. Hydrocortisone acetate and neomycin in otic infections. *The Laryngoscope.* **64**(10): 834-844.
16. HARRIS, H. E. & E. P. LOZA. 1955. The use of neomycin and hydrocortisone in the treatment of external otitis. *Cleveland Clinic Quart.* **22**(1): 10-15.

17. SENTURIA, B. H. & M. D. MARCUS. 1952. Etiologic classification of diseases involving the external ear. *Ann. Otol. Rhinol. & Laryngol.* **61**(1): 18.
18. COLLINS, E. G. 1951. Certain observations on the anatomy, histopathology and physiology of the external auditory meatus. *J. Laryngol. & Otol.* **61**(1): 14-23.
19. MONTAGNA, W. 1949. The glands in the external auditory meatus of the cat. *J. Morphol.* **85**(3): 423-442.
20. SOPHIAN, L. H., Z. K. COOPER & B. H. SENTURIA. 1954. Pathologic changes of the skin of the external auditory canal in chronic otitis media and mastoiditis. *Ann. Otol. Rhinol. & Laryngol.* **63**(2): 261.
21. SENTURIA, B. H. Unpublished data.
22. TAKEDA, S. 1951. Comparative histological examinations of the skin of the external auditory canal. *Okajimas Folia Anat. Japon.* **22**: 295.
23. ROTHMAN, S. 1954. *Physiology and Biochemistry of the Skin*. Univ. Chicago Press. Chicago, Ill.
24. VON TRÖLTSCH. 1903. *Diseases of the Ear*. : 9. By Adam Politzer. Lea Brothers. New York, N. Y.
25. ALZHEIMER, A. 1888. *Über die Ohrenschmalzdrüsen*. *Verhandl. Phys. Med., Würzburg.* **22**.
26. SHELLEY, W. B. & H. J. HURLEY, JR. 1952. Methods of exploring human apocrine sweat gland physiology. *Arch. Dermatol. & Syphilol.* **66**: 156-161; 172-179.

B. EYE

## OCULAR THERAPY WITH THE TOPICAL APPLICATION OF HYDROCORTISONE

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### *Introduction*

A vast literature concerning the experimental and clinical use of cortisone and hydrocortisone has accumulated during the past five years. Despite this fact, there has been very little attempt to correlate the known facts into articles directed at the therapy of specific ocular diseases.

Inasmuch as cortisone was the first adrenal steroid to have been found effective in ophthalmology, more information is available concerning its use than concerning that of hydrocortisone. The evidence available indicates that any clinical or experimental findings may be applied to the use of hydrocortisone. It is generally agreed that any patient who will respond to cortisone will respond equally well, at least, to hydrocortisone. Most observers<sup>1-8</sup> are agreed also that hydrocortisone is superior to cortisone in the treatment of ophthalmic disease.

### PART I

#### EXPERIMENTAL BACKGROUND OF LOCAL THERAPY

Many writers have pointed out that hydrocortisone is superior to cortisone in the treatment of ocular disease. McDonald and Leopold<sup>1</sup> have observed that hydrocortisone has a greater antiphlogistic action than cortisone in the treatment of ocular inflammation. Hydrocortisone has succeeded in cases where cortisone, similarly used, has been unsuccessful.

Gordon<sup>3</sup> found the use of hydrocortisone acetate by subconjunctival injection to be inferior to the similar use of cortisone acetate. Weimar and Leopold<sup>9</sup> have recently gathered experimental evidence showing that hydrocortisone acetate is absorbed less well from the subconjunctival area than cortisone acetate when injected subconjunctivally. In this paper, the term "hydrocortisone" is meant to refer to the use of hydrocortisone acetate unless otherwise specifically stated.

#### *Topical Therapy*

The topical use of steroid therapy in ophthalmology is well grounded, as a result of the clinical experiences of the past five years. Leopold<sup>10</sup> has shown that, as the duration of topical therapy increases, one builds up a higher concentration of cortisone and hence, by inference, of hydrocortisone in the aqueous, with a peak level at approximately 72 hours. With cessation of topical therapy, an immediate drop occurs in the concentration of the steroid in the aqueous. With topical therapy, some cortisone is found in the vitreous,

but never approaching the concentrations found in the aqueous. When the steroid was injected subconjunctivally, he found the maximum level in 24 to 48 hours, with a concentration exceeding that which had occurred with the use of the same steroid topically. The amount found in the vitreous, however, was much higher than with the topical application of cortisone. Increasing the subconjunctivally injected concentrations did not materially increase the intraocular levels. With the greater concentrations, however, the vitreous levels remained higher for a longer period. Leopold did find that an increase in the number of injections was effective. When one eye was injected, he found a slight increase in the intraocular fluids of the uninjected eye. The concentration of cortisone in the aqueous humor after intramuscular injections was equal to that obtained by subconjunctival injections in the dosage schedule that he employed. It required three days to reach the maximum concentration in the vitreous.

Gordon,<sup>3</sup> Leopold<sup>9</sup> and others have pointed out that the white crystals remaining at the sites of subconjunctival injections persist for a much longer period after the injection of hydrocortisone than after the injection of cortisone. Yasuna *et al.*<sup>11</sup> studied the effects of repeated subconjunctival injections of cortisone on the rabbit eye. They revealed that what was formerly thought to be white areas of residual cortisone are, in reality, sterile abscesses incited by the retained cortisone. There were no organisms found in the sections. In the control eyes, which were injected with saline plus the commercial diluent, no such abscesses were found. These investigators conclude that perhaps the use of subconjunctival injections of the steroid in already inflamed eyes may be prejudicial to the interests of those eyes. Hollander and his group<sup>12</sup> have demonstrated that hydrocortisone is much more effective than cortisone in the local therapy of rheumatoid arthritis by direct injection into the joint. This is analogous to the ophthalmologist's subconjunctival injection.

Gordon's<sup>3</sup> clinical experiences with hydrocortisone by subconjunctival injection led him to believe that the free alcohol form of hydrocortisone was much more rapidly effective, although transient in action, than the acetate form, which seemed to get started more slowly but to act over a much longer period. Therefore, he employs a combination of free alcohol hydrocortisone and hydrocortisone acetate for subconjunctival injection, utilizing  $\frac{1}{3}$  to  $\frac{1}{2}$  cc. of the 2.5 per cent concentration at each injection. It is his feeling that the injections act over a period of between 3 to 14 days with an average period of action of approximately 7 to 8 days. Therefore, he employs the subconjunctival injections at intervals of approximately 7 to 14 days, depending upon the response of the patient.

The beneficial effects of topically and subconjunctivally applied cortisone and hydrocortisone indicate that the steroids block inflammatory reaction at the tissue level. There are many cases, especially of posterior ocular involvement, in which subconjunctivally injected hydrocortisone is not beneficial. Many of these cases then respond very well to systemically employed hydrocortisone or ACTH. It is quite possible that the local tissue blocking action of the steroid is not the entire story here. It is possible that systemically em-



ployed hormonal steroid therapy produces results dependent upon mechanisms other than those produced by the purely local action of the steroids.

One finds it difficult to reconcile Weimar and Leopold's<sup>9</sup> statement that, following the cessation of subconjunctival injections of the steroid, the level in the aqueous and vitreous humors begins to drop off rapidly with Gordon's statement that, clinically, a subconjunctival injection is effective approximately seven to eight days, and with the later finding of Yasuna and his group that the white supposed crystalline remnants of the steroid at the site of subconjunctival injection is actually an abscess caused by the retained steroid itself. There are two possible answers to this dilemma: first, that we do not know exactly how much intraocular steroid is necessary for a therapeutic effect. It is probable that the initial high concentration acts to subdue the disease reaction, while the residual decreasing "depot" of steroid acts to keep it suppressed. It is likely that a steroid level that is below that which can be detected by laboratory test is effective clinically. Second, it may be that if, as Yasuna claims, the local deposit is an abscess rather than simply a mass of steroid crystals alone, the "abscess" itself has some beneficial effect. This speculation is interesting. It is possible that both of these answers are operative here. The author has many cases that have received between 25 and 50 subconjunctival injections of cortisone and hydrocortisone over a period of 2 to 3 years without any apparent deleterious effect upon the eye. There is not the slightest bit of clinical evidence to indicate that the use of subconjunctival injections of the steroid in an already inflamed eye has other than a beneficial effect.

### *Wound Healing*

Much of the experimental work done, especially in the early days of cortisone therapy, employed massive doses. These doses were often as much as 25 times in excess of the normal clinical dose. Nevertheless the results of these experiments were reported and, as often happens, the readers overlooked the massiveness of the dose and tended to apply these results directly to clinical management. The early work<sup>13</sup> concerning the interference of massive doses of corticosteroids with wound healing threw the profession into confusion. Many people still fear to employ cortisone or hydrocortisone in the presence of a wound area.

The experimental evidence indicates that there is, if anything, but slight retardation of epithelial regeneration of corneal abrasions. It is generally agreed that, with ordinary clinical dosages of topically applied steroid, no harmful effect may be expected upon regeneration of corneal epithelium. James, Power, and Ripple<sup>14</sup> found that the tissue reaction of the corneal stroma, fibroblastic proliferation, and lymphocytic infiltration were retarded by subconjunctival injections of cortisone in much more massive doses than are employed clinically. Foreign body reaction about the sutures was slight in the cortisone-treated eyes in comparison with control eyes.

It is generally agreed by research workers in the field that steroids inhibit cellular infiltration and fibroblastic activity associated with inflammation as well as the formation of granulation tissue in the eye and in the body generally. Hydrocortisone has a strong inhibitory effect on the formation of

fibrinous exudate, the ingrowth of fibroblasts, and the cellular infiltration usually found in the healing of corneal wounds (McDonald, Leopold, *etc.*<sup>15-20</sup>).

It would appear that, in corneal abrasions that are not infected, hydrocortisone is very beneficial and epithelialization is not hindered. Rather, the lack of stromal activity in certain inflammations favors early coverage of the wound.

Most writers are in agreement that the beneficial effect of hydrocortisone upon ocular pain is very welcome indeed. Yasuna found a slight increased healing in abrasions in cortisone-treated animals as compared to nontreated animals, but considered this amount within normal limits.

Newell<sup>17</sup> found that, with much larger than normal doses, he delayed healing in corneal transplants in rabbits

The available evidence indicates that cortisone, in therapeutic doses, has no inhibitory effect upon tissue cultures. Steen<sup>21</sup> found that it took 25 times the usual therapeutic level to inhibit tissue growth in cultures. Ashton and Cook<sup>16</sup> found the amount of inhibition directly related to the amount of cortisone employed.

### *Corneal Abrasions*

There is general agreement that, in normal clinical doses, topically applied hydrocortisone minimizes the scarring in corneal abrasions. The author has found, over a period of five years, that the use of cortisone and, later, hydrocortisone, following foreign bodies of the cornea, resulted in appreciably less scarring than in the presteroid days. This impression is purely a clinical one. He has had a number of cases of metallic foreign bodies, however, in which he has been unable to scrape away as much of the corneal metallic staining as he would have liked. In none of these cases, which were treated with topically applied steroids, have the patients encountered any difficulty. It was the clinical impression, in several of these, that the steroid saved the patient and physician complications.

The role of hydrocortisone in the treatment of severely lacerated eyes is not clear-cut, at this moment. The number of preparations employed in any one such case renders it almost impossible to evaluate the efficacy of the steroid here.

### *Bullous Keratitis*

Despite some earlier reports of success with the use of cortisone in the treatment of traumatic bullous keratitis, this author has had, essentially, no success in treating a large number of cases with either cortisone or hydrocortisone.

### *Striate Keratitis*

Evaluation of the efficacy of any preparation in the treatment of striate keratitis, a condition that, in practically every case, clears spontaneously is rather difficult. It has been this author's impression, however, that in some very severe postoperative cases, the topical applications of hydrocortisone have been beneficial. Topical applications of the steroid have paled the eye more rapidly than would have occurred spontaneously. While some physicians question the advisability or necessity of paling the eye rapidly, there is no question but that the patient is happier and much more comfortable.

*Burns*

There is general agreement that the early employment of the steroids in the treatment of corneal burns is advisable and beneficial. Leopold<sup>10</sup> pointed out that, while there was slight retardation of epithelial regeneration in thermal burns of the cornea, the density of the resultant cicatrix was less with the steroid-treated eyes, regardless of the route of administration. He felt that systemic therapy was less efficacious than local steroid therapy here. In acid burns of the cornea, he found that the density of the scar was benefited also by cortisone by any route. In alkali burns of the cornea, the density of the cicatrix was slightly reduced by topical cortisone. The day of onset of vascularization, however, was delayed, and the number and length of the invading vessels into the cornea was reduced. It is interesting that, in this experiment, not one of the eyes treated by cortisone was a victim of a secondary infection.

Leahy<sup>22</sup> uses 0.5 per cent cortisone solution at each dressing of corneal and conjunctival burns and, later, four times daily, when the dressings have been removed. He found that, on corneal wounds especially, the steroid cut down the irritability of the eye and consequently the tendency for the leukoma to vascularize. In extensive burns, systemic steroid or ACTH for the first few days appears to reduce the severity of inflammatory reaction and thus lessens the tendency for burn shock.

McLaughlin,<sup>23</sup> in treating chemical burns, converts all of these burns into mechanical chemical injuries by denuding all of the involved corneal epithelial cells. He then employs the steroid in ointment and feels it of value in preventing vascularization of the cornea following these extensive ulcers. It might be well to state, at this point, that iritis is a very common complication of chemical burns. Hence, the employment of hydrocortisone here would be directed at both the keratitis and the uveitis. One can readily appreciate the beneficial effect upon the ocular pain accompanying corneal burns.

It would appear obvious that hydrocortisone has a definite role in the treatment of corneal burn. It should be employed early, and as often as necessary without unduly disturbing the eye.

*Neovascularization*

Beneficial effects of topically applied steroid therapy upon corneal neovascularization<sup>24</sup> have been known almost from the first days of cortisone and hydrocortisone. Jones and Meyer<sup>25</sup> pointed out these effects very early with the use of topical cortisone. Michaelson<sup>26</sup> has been greatly interested in the subject of corneal neovascularization. He has demonstrated that subconjunctivally injected cortisone reduced the extent of the standard vascular area by approximately 45 per cent. He found that employment of 0.5 per cent cortisone by topical instillation was as good as subconjunctivally injected cortisone. He felt that when he employed 1.25 mgm. of cortisone by subconjunctival injection, the effect was essentially the same as when he employed twice that dosage. That conclusion is in line with the results of Leopold's work on the use of subconjunctivally injected steroid. Gordon has seen at least one case in which topically applied cortisone and, later, hydrocortisone were not particularly effective in reducing an intense localized area of neovascularization

into the cornea. Once hydrocortisone was injected subconjunctivally at the site, however, the neovascularization was rapidly brought under control.

### *Corneal Ulcers*

There is no clear-cut agreement on the use of hydrocortisone with or without an antibiotic in the treatment of infected corneal ulcers. It is known that hydrocortisone itself has no therapeutic effect upon bacteria. This being the case, it is obvious that, if hydrocortisone is going to be used in the treatment of an infected cornea, it should be in conjunction with an antibiotic. Even here, there is no clear-cut agreement. It is this author's feeling that, in the presence of an infected cornea, the combination of the anti-inflammatory hydrocortisone and an antibiotic is beneficial. This impression, however, is only a clinical one unsupported by any laboratory evidence. The writer has seen some patients in whom neither an antibiotic nor the steroid alone were beneficial, but in whom the two, used together, caused healing of the ulcerated area. In this connection, it may be of interest to note that this author served as a consultant several years ago in several of the cases that developed keratitis following the use of a steroid preparation contaminated with pyocynin. In all but one of these cases, the steroid was discontinued immediately, and the patient was treated with antibiotics. In not one of this group did a good result occur. It is noteworthy that the only one of this series which recovered pretreatment vision was one on whom steroid therapy was continued with a sterile preparation in combination with antibiotic treatment. An excellent result occurred.

Lepri<sup>27</sup> found that cortisone *in vitro* had no inhibitory effect upon the growth of any of the organisms examined, confirming the earlier findings of other authors. "When cortisone is combined with antibiotics there is an attenuation of the inflammatory signs not apparent in those animals which receive antibiotic treatment only (penicillin or terramycin). Perikeratic congestion is less evident, there is less pain with pressure on the ciliary body, and congestion of the iris decreased." The duration and outcome, however, was no different than in the animals who received antibiotics alone. The more rapid paling and the increased comfort to the patient is stressed.

Leopold<sup>28</sup> feels that, in cases of potential infection, one should either avoid the use of the steroid until certain that no infection will develop, or not employ antibiotics that depend for their intraocular concentration upon the increased permeability associated with trauma or inflammation. He found that chloramphenicol and streptomycin were effective when employed in conjunction with steroid therapy.

### *Surgery*

The introduction of hormonal steroid therapy has rendered it possible to operate upon a great number of eyes which otherwise could not be brought to surgery. One here refers especially to patients suffering from chronic uveal inflammation, who can be operated upon while under such a regime. A vast clinical and experimental experience<sup>29</sup> has been acquired which indicates that it is safe to operate upon a patient who is receiving normal clinical doses of hydrocortisone, without any deleterious effect upon the corneal wound healing.



Yasuna and his co-workers, in their experimental studies of rupture of corneal wounds, found that there was no significant difference between treated and control animals. They felt that, after the sixth day of healing, the cortisone-treated group lagged behind the untreated group, but not greatly so.

Thorpe<sup>30</sup> found no interference with ocular wound healing in his series of cases. Laval,<sup>31</sup> who employed cortisone subconjunctivally once weekly after glaucoma surgery in rabbits, found that the treatment had no effect in decreasing the amount of granulation tissue which forms, or on the degree of fibrosis which took place. When he employed large doses intramuscularly, however, he found inhibition.

At the New York Hospital, we have operated in the last five years upon a large number of patients who were under hormonal steroid therapy, either topically or systemically, with no evidence of deleterious effect. One patient did receive considerable cortisone topically, starting with the third day after cataract extraction, and exhibited gaping of the wound edges after discharge. When the cortisone was stopped, the wound healed normally. This case, however, was only one in a large series. The preponderance of evidence indicates that hydrocortisone, employed in normal clinical amounts, has no deleterious effect upon corneal wound healing, following intraocular surgery.

As has been stated, this author has found no beneficial effect from the employment of hydrocortisone in the treatment of postoperative bullous keratitis. He does, however, feel that hydrocortisone may be beneficial in the treatment of severe striate keratitis following intraocular surgery. Most patients always exhibit considerable pericorneal congestion and also ocular pain, which is definitely relieved by the topical applications of the steroid. This relief justifies its use. Holland and Lepisto<sup>32</sup> found that steroid therapy had no apparent influence on striate keratitis, although it did relieve postoperative discomfort and shortened hospital stay after cataract surgery.

### *Keratoplasty*

Woods<sup>33</sup> states that a number of cases of corneal transplants associated with early clouding and vascularization of the graft have been treated with cortisone topically as well as by systemic hormonal-steroid therapy. Treatment with these agents usually was begun about the second to fifth week after surgery and after clouding or neovascularization of the graft had already taken place. When therapy was begun within the first three days after clouding was observed, approximately one half or more of the patients exhibited a clearing of the graft with decrease of vascularization, converting what threatened to be an unfavorable result into a favorable one. Woods felt that topical steroid appeared to be the preferable method of treatment. This therapy, however, must be begun before the changes are established and irreversible. Maumenee's<sup>34</sup> work has indicated that delayed clouding of the graft may be related to an allergic reaction to the donor graft by the patient. The known beneficial effects of hydrocortisone in treating allergic conditions would render it indicated in these cases. It is Woods's feeling that treatment should be instituted early in keratoplasty; that it is best not started until about two weeks after the operation in order that there be no interference with firm union of the graft.

Paton<sup>35</sup> uses hydrocortisone topically, starting at the time of removal of the sutures and continuing until the eye is completely pale. He finds it beneficial in early clouding of the cornea and in impeding vascularization of graft.

McLean<sup>36</sup> employs topical therapy, starting at about the third postoperative day, with each dressing. After the bandage is removed, the patient uses it several times daily.

The weight of evidence is in favor of topical hydrocortisone as a beneficial agent in the management of keratoplasty.

## PART II

### TOPICAL AND LOCAL HYDROCORTISONE IN UVEITIS

Hormonal-steroid therapy has revolutionized the treatment of uveitis. The role of hydrocortisone here is that of a newer compound replacing older proved ones where the former fail, or where the newer one can be employed more efficiently.

The vast clinical and experimental data secured with cortisone may be applied to the use of hydrocortisone. McDonald and Leopold<sup>1</sup> found that, while most of their patients with anterior uveitis could be controlled with topical cortisone, some who did not could be controlled with topical hydrocortisone.

The author has at least one case of generalized uveitis which is made worse with subconjunctival or systemic cortisone. This patient did very well on corticotropin given over a long period. For over two years now, he has been controlled on subconjunctival hydrocortisone (free alcohol plus acetate) given every several weeks.

Laval<sup>5</sup> had two cases of severe keratitis and uveitis. One failed on cortisone and corticotropin combined, and the other on cortisone systemically. Both did well on subconjunctival hydrocortisone acetate combined with the same topically.

At least five cases of glaucoma secondary to uveitis have been controlled on hydrocortisone alone or in combination with Diamox and/or other topical therapy (Gordon).

Vogel and Leopold<sup>37</sup> reported that "compound F (7.5 mgm. daily) injected subconjunctivally seven days after horse serum had been placed intravitreally modified markedly the primary anaphylactic horse serum uveitis in rabbits." The hydrocortisone, employed in this fashion, inhibited the formation of chemosis, anterior chamber ray, and keratic precipitates after an intravitreal shocking dose of horse serum. It prevented chemosis to a more marked degree than did intramuscular hydrocortisone. It might be pointed out here that this statement must not be taken to indicate a superiority of the subconjunctival route over the intramuscular. It is a fact that we still do not know what amount of hydrocortisone subconjunctivally is equivalent to what amount systemically. It is also a fact that, in some patients, one route is more effective than another for reasons which are not always clear.

It is a matter of common experience that a very high percentage of cases with acute anterior uveitis, especially if seen early, will respond to topical hydrocortisone given approximately every two hours initially, with increasing

intervals as the disease responds. The author has spoken to many ophthalmologists whose experiences have confirmed this finding. Practically all of these practitioners employ atropine or some other mydriatic simultaneously. It is a fact that many cases of anterior uveitis will respond to the latter alone, although not as rapidly or as comfortably as to hydrocortisone.

Many cases which fail on the above regime will do well on subconjunctivally injected hydrocortisone, preferably (in this author's experience) if the combination of free alcohol and acetate is utilized. Injections are repeated at intervals of three to seven days initially, dependent upon the response of the inflammation. In acute cases, it is rare to require more than two to three such injections.

In all fairness, despite the fact that the title of this monograph limits the author to a discussion of the uses of topically employed hydrocortisone (and the word "topical" has been conveniently stretched to mean "local") some mention of the author's position must be made.

#### *Acute Anterior Uveitis*

It is impossible to gauge the potential severity and duration of any given case of acute anterior uveitis (or recurrent anterior uveitis) when first seen. Because of that, and because of the simplicity and safety of short courses of systemic therapy and the proven superiority of systemic administration as opposed to topical, this writer regards every case of uveitis as potentially serious. He plans on several days of systemic hormonal-steroid therapy in addition to the topically applied material in initiating treatment. In the average case (especially if it is an early one) most patients are sign- and symptom-free before the fourth day, and are then continued for an additional approximate two weeks on topical therapy. Cycloplegia is not employed unless synechiae are already present. In the latter event, short acting compounds as cyclogyl and phenylephrine hydrochloride are used just long enough to serve their purpose. The systemic therapy seems to help melt the synechiae. This regime is an ambulatory one, permitting the patient to continue working with two pupils of essentially equal size.

In recurrent cases, the course is often a more stormy one. While many of these may respond to topical hydrocortisone, it would seem advisable to shorten their course as much as possible by employing subconjunctival or systemic therapy.

#### *Chronic Uveitis*

The management of the chronic case of uveitis poses a real problem. Here, it is the author's experience that topical hydrocortisone plays only a supporting role to systemic therapy. In some of these cases, subconjunctival hydrocortisone ( $\frac{1}{3}$  to  $\frac{1}{2}$  cc. of the 2.5 per cent suspension) may function either as primary therapy or as partial replacement for systemic therapy. The experience of five years testifies that there is virtually no reasonable limit to the number of subconjunctival injections that any one patient may receive. A number of one-eyed patients, the other eye lost to chronic uveitis, have been kept ambulatory and employable on a regime employing systemic, subconjunctival and topical therapy.

The question of differentiating between granulomatous and nongranulomatous uveitis may be brought up here. Without going into an involved discussion of these two main types of uveitis, the classification would not seem overly important as far as steroid management is concerned. Certainly, the granulomatous cases tend to be more difficult and more chronic. If a specific therapy is at hand, it should be the treatment of preference. One must admit that this is rarely the case. Experimental evidence would indicate that hydrocortisone would be dangerous in tuberculous uveitis. Where this is the presumptive diagnosis, one should perhaps employ other antituberculous therapy without or with hydrocortisone. On the other hand, if tuberculous uveitis is as prevalent as we have been led to believe, it is interesting that the literature has not been noteworthy for case reports citing complications as a result of steroid management in such cases.

#### *Sympathetic Ophthalmia and Phacoanaphylactic Uveitis*

The bulk of the literature on these subjects has concerned itself with prehydrocortisone days. Hormonal-steroid therapy has been of great aid in some of these earlier cases. A review of the literature plus personal experience and conversations with other ophthalmologists stimulates the belief that hydrocortisone, when applied topically, is valuable here in a supporting role only. Purnell and Leopold's<sup>38</sup> experiences with subconjunctival therapy in phacoanaphylactic uveitis would indicate that this route offers a 50-50 chance of success. It is difficult to give credence to reports of success with topical steroids in acute active cases, unless these were extremely mild. There is no question but that topical hydrocortisone will rapidly pale and render more comfortable an eye which still contains an active uveitis. If one does not employ a slit-lamp, this can be misleading.

#### *Subconjunctival Hydrocortisone*

The subconjunctival route, employing  $\frac{1}{3}$  to  $\frac{1}{2}$  cc. of 2.5 per cent suspension offers a convenient method of creating a depot of steroid. Where topical therapy alone cannot (or cannot be expected to) carry the burden alone, this avenue may serve the purpose. Subconjunctival hydrocortisone is cheaper and subject to fewer complications than systemic therapy, and can make for partial or complete substitution for the latter. It is especially convenient in patients who cannot be trusted to obey orders, or who must be out of the ophthalmologist's sight for some days, and in whom there is fear of side effects from systemic therapy. It is especially valuable in patients who have peptic ulcers or some other contraindication to systemic therapy with either cortisone, hydrocortisone, or corticotropin.

#### *9-Alpha-Fluorohydrocortisone\**

The author has had the opportunity to treat a fairly large series of patients with this new preparation. Employed topically as a drop, or ointment, it will apparently do everything that hydrocortisone (acetate or free alcohol) will do. It can be employed in  $\frac{1}{10}$  to  $\frac{1}{20}$ th the strength of hydrocortisone in the same

\* This preparation was supplied through the courtesy of Doctor H. Thomas of Sharp & Dohme, Philadelphia, Pa.



type case. Since it is in solution, it is less irritating. In some instances, it appears to be more effective in the treatment of chronic cases who have employed hydrocortisone formerly.

The question arises as to whether its solubility will make for greater penetration into the globe when employed topically, and, if that is so, if employment of much higher concentrations will render it effective in conditions where subconjunctival or systemic therapy must now be employed. This question will be explored.

### *Conclusions*

(1) The known effects of hydrocortisone in reducing corneal stromal activity, fibroblastic proliferation, granulation tissue formation, lymphocytic infiltration, and neovascularization render it valuable in the therapy of corneal abrasions, burns, and in the management of keratoplasties.

(2) The beneficial effects of hydrocortisone upon ocular pain and inflammation (as well as on allergies) indicate its use in the postoperative management of many cases. In some cases, patients with chronic uveitis would have to be denied surgery were it not for the availability of these agents.

(3) Hydrocortisone has played a prominent role in revolutionizing the treatment of uveitis. Topical therapy has its most important application in the therapy of acute anterior uveitis. In the more severe and chronic uveitides, it plays a supporting role.

(4) The place of subconjunctival hydrocortisone therapy is stressed.

(5) The new 9-alpha-fluorohydrocortisone has proved effective by the topical route in conditions in which hydrocortisone is known to be beneficial. Experience should further delineate its exact role in the ophthalmic armamentarium.

### *References*

1. McDONALD, P. R., I. H. LEOPOLD, A. W. VOGEL & R. D. MULBERGER. 1953. Hydrocortisone (compound F) in ophthalmology. *Arch. Ophthalmol. Chicago*. **49**(4): 400.
2. GORDON, D. M., J. M. McLEAN & H. KOTEN. 1953. Present status of corticotropin (ACTH), cortisone and hydrocortisone in ophthalmology. *Brit. J. Ophthalmol.* **37**: 85.
3. GORDON, D. M. 1954. Hydrocortisone in ophthalmology. *Am. J. Ophthalmol.* **37**(4): 533.
4. GORDON, D. M. 1954. The Clinical Use of Corticotropin, Cortisone and Hydrocortisone in Ophthalmology. Thomas. Springfield, Ill.
5. LAVAL, J. 1953. Use of hydrocortisone (hydrocortisone acetate) in ophthalmology. *Arch. Ophthalmol. Chicago*. **50**(3): 299.
6. STEFFENSEN, E. H. 1952. Corticotropin, cortisone and hydrocortisone in the treatment of ocular disease. *J. Am. Med. Assoc.* **150**: 1660.
7. STEFFENSEN, E. H., H. B. IVY & F. O. NAGLE. 1952. Topical compound F in the treatment of anterior segment eye disease. *Am. J. Ophthalmol.* **35**(7).
8. DUKE-ELDER, W. S. 1951. The clinical value of cortisone and ACTH in ocular disease. *Brit. J. Ophthalmol.* **35**: 637.
9. WEIMAR, V. L. & I. H. LEOPOLD. 1954. Intraocular penetration of local hydrocortisone and cortisone. *Arch. Ophthalmol. Chicago*. **52**(5): 769.
10. LEOPOLD, I. H. & F. R. MAYLATH. 1952. Intraocular penetration of cortisone and its effectiveness against experimental corneal burns. *Am. J. Ophthalmol.* **35**(8): 1125.
11. YASUNA, J. M., A. W. OJERS, W. C. FRAYER & H. G. SCHEIE. 1954. An experimental study of the effect of cortisone on the eye. **37**: 923.
12. HOLLANDER, J. L., E. M. BROWN, JR., R. A. JESSAR & C. Y. BROWN. 1953. Hydrocortisone and cortisone injected into arthritic joints: comparative effects of and use of hydrocortisone as a local antiarthritic agent. *J. Am. Med. Assoc.* **147**: 1629.

13. RAGAN, C., F. L. HAWES, C. M. PLOTZ, K. MEYER & J. W. BLUNT. 1949. Effect of cortisone on production of granulation tissue in the rabbit. *Proc. Soc. Exptl. Biol. Med.* **72**: 718.
14. JAMES, W. H., J. L. POWER & P. H. RIPPLE. 1952. Experimental and clinical observations of the effects of cortisone in corneal lesions. *Am. J. Ophthalmol.* **35**(9): 1298.
15. LEOPOLD, I. H., J. E. PURNELL, E. J. CANNON, C. G. STEINMETZ & P. R. McDONALD. 1951. Local and systemic cortisone in ocular disease. *Am. J. Ophthalmol.* **34**: 365.
16. ASHTON, N. & C. COOK. 1951. Effect of cortisone on healing of corneal wounds. *Brit. J. Ophthalmol.* **35**: 708.
17. NEWELL, F. W. & J. M. DIXON. 1951. Effect of subconjunctival cortisone upon the immediate union of experimental corneal grafts. *Am. J. Ophthalmol.* **34**: 977.
18. COLE, J. W., J. L. ORBISON, W. O. HOLDEN, T. J. HANCOCK & J. F. LINDSAY. 1951. A histological study of the effect of cortisone on wound healing per primam. *Surg. Gynecol. Obstet.* **93**: 321.
19. DUKE-ELDER, W. S. & N. ASHTON. 1951. Action of cortisone on tissue reactions of inflammation and repair with special reference to the eye. *Brit. J. Ophthalmol.* **35**: 695.
20. CORNION, I. 1951. Selective damage to fibroblasts by desoxycorticosterone in cultures of mixed tissue. *Science*. **113**: 37.
21. STEEN, S. A. 1951. Effect of cortisone on tissue cultures. *Brit. J. Ophthalmol.* **35**: 741.
22. LEAHY, B. D. 1952. Thermal burns of the eye and adnexa. *Am. J. Ophthalmol.* **35**(8): 1077.
23. McLAUGHLIN, R. J. 1952. Chemical burns of the cornea. *Am. J. Ophthalmol.* **35**(8): 1088.
24. LISTER, A. & D. P. GREAVES. 1953. Effect of cortisone upon the vascularization which follows corneal burns. *Brit. J. Ophthalmol.* **35**: 725.
25. JONES, I. S. & K. MEYER. 1950. Inhibition of vascularization of the rabbit cornea by local application of cortisone. *Proc. Soc. Exptl. Biol. Med.* **74**: 102.
26. MICHAELSON, I. C. 1952. Comparison of results of cortisone treatment by instillation and by subconjunctival injection. *Arch. Ophthalmol. Chicago*. **48**: 144.
27. LEPRI, G. Studies on cortisone in ophthalmology. I. Influence of cortisone on the *in-vitro* effect of antibiotics on microorganisms capable of causing ocular infections. II. The action of cortisone on experimental pneumococci infection of the cornea and its influence on the activity of several antibiotics.
28. LEOPOLD, I. H. 1952. Surgery of ocular trauma. *Arch. Ophthalmol. Chicago*. **48**(6): 738.
29. SMITH, R. L., R. M. FASANELLA, E. ROSENTHAL & I. E. HOFFMAN. 1954. Cortisone in lens dissection and extracapsular extraction. *Arch. Ophthalmol. Chicago*. **52**(4): 545.
30. THORPE, H. E. 1951. ACTH and cortisone in ocular surgery. Joint Meet. Sect. Ophthalmol. Am. Med. Assoc. & Assoc. Research Ophthalmol., June 15th.
31. LAVAL, J. & R. S. COLES. 1953. Role of cortisone in glaucoma surgery. *Arch. Ophthalmol. Chicago*. **49**(2): 168.
32. HOLLAND, R. W. B. & V. E. LEPISTO. 1954. Cortisone and neosone in complications following cataract surgery. *Am. J. Ophthalmol.* **38**(2): 201.
33. WOODS, A. C. 1954. Medical uses of cortisone (including hydrocortisone and corticotropin). *In Eye Diseases*. F. D. W. Lukens, Ed. Blakiston. New York, N. Y.
34. MAUMENEE, A. E. 1951. The influence of donor-recipient sensitization on corneal grafts. *Am. J. Ophthalmol.* **34**(5, part 2): 87.
35. PATON, T. Personal communication.
36. McLEAN, J. M. Personal communication.
37. VOGEL, A. W. & I. H. LEOPOLD. 1953. The effect of compound F upon horse serum uveitis in the rabbit eye. *Am. J. Ophthalmol.* **36**(5): 690.
38. PURNELL, J. E. & I. H. LEOPOLD. 1952. Cortisone in ocular disease: further studies. *Am. J. Ophthalmol.* **35**(5, part 1): 663.

# THE USE OF TOPICAL HYDROCORTISONE IN THE TREATMENT OF INFLAMMATORY LESIONS OF THE CORNEA, SCLERA, AND CONJUNCTIVA

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Corticotropin, cortisone, and, more recently, hydrocortisone have been extensively used in the treatment of a variety of ocular lesions, and, in many cases, the therapeutic effects have been most striking.

The initial experimental use of these agents in ophthalmology was not without a rational basis. This work began late in 1949, first with corticotropin. From studies available at that time, it appeared that the beneficial effect of fever therapy so commonly used in the treatment of a variety of ocular lesions was achieved through the endogenous release of corticotropin.<sup>1</sup> It therefore appeared logical to give corticotropin directly, where the effect could more accurately be controlled.

When it was shown that corticotropin gave results superior to fever therapy, it seemed reasonable that one of the 11-oxycorticosteroids, such as cortisone, would give similar results. This finding is now well established, and, to date, no essential difference has been noted in the favorable action of the two drugs during short-term parenteral administration.<sup>2</sup>

It was then postulated that cortisone acted at the local tissue level and that it would have physiological activity when applied topically.<sup>3</sup> That postulate proved true and, in most lesions involving the anterior segment of the eye, topically administered cortisone has given results comparable to those achieved with parenteral administration.

Hydrocortisone acetate suspensions and ointment have been used topically for the past four years. Since cortisone is so effective in a variety of anterior segment eye lesions, it is difficult to judge the relative merits of the two drugs clinically. So far, no selective activity has been noted. That is, no ocular lesion will respond to the one drug and show no response to the other. Though laboratory studies by McDonald and his co-workers<sup>4</sup> failed to show any significant difference between topically administered cortisone and hydrocortisone, several clinical reports indicate that hydrocortisone is slightly more effective.<sup>4-7</sup> It is interesting that the clinical response to the two is so similar in ocular disease, whereas, when the drugs are applied locally to the skin, the superiority of hydrocortisone over cortisone is pronounced.

The merits of administering hydrocortisone topically are obvious. Such treatment is relatively inexpensive and does not necessitate hospitalization. There is no danger of systemic effect, regardless of the duration of treatment. Topically administered hydrocortisone can be given either in the form of ointment or suspension. Two strengths of the suspension are available commercially, 0.5 per cent and 2.5 per cent. In most cases, the 0.5 per cent suspension is adequate. The frequency of administration depends upon the severity of the disease process and varies from every hour to once or twice a day.

TABLE 1

ANTERIOR SEGMENT EYE LESIONS THAT RESPOND TO TOPICAL HYDROCORTISONE

---

Allergic conjunctivitis
Allergic blepharoconjunctivitis
Vernal conjunctivitis
Episcleritis
Nodular scleritis
Keratoconjunctivitis
Phlyctenular
Marginal ulcer
Keratitis
Tuberculous
Sclerosing
Acne rosacea
Profunda (nonspecific)
Superficial (nonspecific)
Syphilitic interstitial
Disciform
Burns, chemical or thermal

---

Brief mention should be made of the subconjunctival injection of hydrocortisone in the treatment of inflammatory lesions of the cornea, sclera, and conjunctiva. In the main, this mode of administration has been discontinued, primarily because it adds inflammatory reaction to an already inflamed eye. Like cortisone,<sup>8</sup> it frequently causes sterile subconjunctival abscesses. Subconjunctival injections are no more effective than the frequent administration of the suspension or the ointment in the treatment of anterior segment eye disease.

TABLE 1 lists the more commonly encountered lesions of the cornea, sclera, and conjunctiva that show a favorable response to topical hydrocortisone.

To the man in other branches of medicine, it is often surprising when ophthalmologists confess that, frequently, they are not certain of the etiological factor for a number of the ocular lesions that respond favorably to hydrocortisone. Some of these lesions are truly allergic. Others may be caused by focal infection mediated through a hypersensitivity in ocular tissues. All that respond have an inflammatory component. Hydrocortisone has no effect upon purely trophic or degenerative lesions of the eye. Since our knowledge as to the etiology of the ocular lesions is incomplete, from this standpoint we have nothing to add to the present concepts concerning the action of hydrocortisone on body tissues. Generally, the beneficial effect of this corticosteroid can be explained by its known properties. It appears that the fundamental principles do not differ from those that act in suppressing inflammatory reactions in other parts of the body. Hydrocortisone apparently has no unique action on ocular tissues.

Cortisone does not generally alter the production of antibodies to sensitizing antigens,<sup>9</sup> nor does it inhibit the antigen-antibody reaction.<sup>10</sup> However, it markedly suppresses this reaction. Experimentally, cortisone blocks the allergic response in cases of so-called "delayed" allergic reaction. By that is meant the allergic response not directly due to immediate histamine release. Experimentally, cortisone does not appreciably alter the tissue effect of allergic



responses presumably due to histamine release.<sup>11</sup> Clinically, however, cortisone and hydrocortisone appear effective in some allergic conditions, such as allergic conjunctivitis due to pollen sensitivity, where a histamine type of response is apparently present.

It is well known that one of the general properties of hydrocortisone is to inhibit inflammation, allergic or otherwise. This nonspecific inhibition of inflammation undoubtedly is most important, but the mechanisms by which it is accomplished are still being investigated. The basic mechanism may well be the ability of hydrocortisone to restore an abnormal capillary permeability to its normal state. This is probably achieved by inhibiting the activity of leukotaxine, and thus preventing the increase in capillary permeability and cellular migration that this substance normally produces.<sup>12</sup>

The ability of cortisone to suppress neovascularization was one of the early properties reported from topical use in the eye.<sup>13</sup> Though laboratory studies indicate that hydrocortisone is less effective in this respect,<sup>4</sup> clinically, no difference has been detected. Corneal vascularization can be a severe and crippling complication of any of the corneal lesions listed in TABLE 1. Topical hydrocortisone, or cortisone, by suppressing the formation of new vessels, has done much to preserve good visual acuity in these cases.

It is well known that hydrocortisone has the property of suppressing fibroblastic proliferation, and this has done much to prevent symblepharon formation in either chemical or thermal burns. In these cases, hydrocortisone therapy has led to clearer corneas, partly through decreased scarring and partly through diminution of the neovascularization, which so commonly occurred with other forms of therapy.

One must emphasize that it is still considered the "normal pattern" for certain ocular lesions to be recurrent. The favorable initial response of such a lesion to topically administered hydrocortisone in no way suggests that a future relapse will be prevented. This hormone only prevents or minimizes the destructive phase of inflammation. Certain ocular lesions appear to be self-limited and, if the destructive phase can be inhibited until the disease runs its course, marked loss of vision may be prevented.

One cannot be too emphatic in noting that, in disease due to allergy or to other specific etiological factors, concurrently the appropriate treatment must be directed toward the eradication of the basic cause. Again, for years, focal infection in the body has been widely accepted as a possible etiological factor in certain inflammatory eye diseases, mediated through a hypersensitivity in ocular tissues. Certainly, that concept cannot yet be discarded. In such cases, topical hydrocortisone would be effective therapy by minimizing the hypersensitivity reaction. Later, relapse could be prevented only by eradication of the focal infection. It should be pointed out here that hydrocortisone, like cortisone, reduces the body defense mechanisms to bacterial invasion. It inhibits the protective inflammation. Though apparently it does not decrease phagocytosis, phagocytosed bacteria are more difficult to destroy.<sup>14</sup> Thus, hydrocortisone should not be used in lesions that are known to be bacterial or viral in origin unless an effective antibiotic is given concurrently.

With the prolonged use of parenteral hydrocortisone, there are dangers of severe adverse pharmacological side effects. When administered topically, the amounts of hydrocortisone acetate used are so small that there is no danger of systemic effect, regardless of the duration of treatment. It should also be mentioned that, when properly used, there is no danger of harmful local effects on ocular tissues. In certain cases, prolonged use of local hydrocortisone causes stippling of the corneal epithelium, which disappears immediately after the drug is discontinued. This mild irritative effect is apparently caused by the vehicle in which hydrocortisone is suspended. In a few cases, where treatment has been prolonged for many weeks, it has been noted that surgical procedures are more difficult, due to the possible softening of the connective tissue of the sclera and cornea.<sup>15</sup> Such an effect has not been noted by slit-lamp studies. In open corneal lesions, topical hydrocortisone in moderate dosages suppresses neovascularization and fibroblastic proliferation without measurable effect upon the regeneration of the corneal epithelium. Heavier dosages, however, appear to retard epithelization slightly. Hydrocortisone has no measurable effect upon pupillary reaction or accommodation, nor upon normal intraocular pressure. It should be emphasized again that serious sequelae can result from the use of hydrocortisone in anterior segment eye lesions that are viral or bacterial in origin. If an effective antibiotic is available to treat the infectious process, then the concurrent use of the hormone may be justified to minimize the destructive inflammatory processes.

Clinically, it is apparent that the therapeutic action of hydrocortisone is semiquantitative. The more severe the inflammatory lesion, the larger are the quantities of this corticosteroid necessary to give therapeutic benefit. This is well illustrated in cases of granulomatous scleritis that respond poorly to topical administration, due to the limited penetration into the sclera, but respond rapidly to the parenteral administration of the drug.

Especially in corneal lesions, we have repeatedly observed rapid relief of pain and photophobia, even in patients who later showed no definite objective improvement. Clinical studies have failed to show that this relief results from any measurable amount of corneal anesthesia. One cannot say whether this apparent analgesia is another nonspecific property of hydrocortisone, or whether it merely reflects the action of hydrocortisone on the inflammatory process. This same response has been noted before with topical cortisone.<sup>16</sup>

In the past, a variety of preparations of hydrocortisone have been tried clinically on anterior segment eye lesions. The most promising of these is 9- $\alpha$ -fluorohydrocortisone acetate. Aside from its equal effectiveness in lower concentrations, as yet, there appears to be no other advantage in using this newer preparation. The 9- $\alpha$ -fluorohydrocortisone acetate suspensions and ointment elicit more subjective responses of irritation. As yet, no local harmful effects have been noted, nor have there been any systemic reactions from its topical use in the eye.

### References

1. OLSON, J. A., E. H. STEFFENSEN, R. R. MARGULIS, R. W. SMITH & E. L. WHITNEY. 1950. Effect of ACTH on certain inflammatory diseases of eye: preliminary report. *J. Am. Med. Assoc.* **142**: 1276-1278.

2. OLSON, J. A. *et al.* 1951. Use of adrenocorticotrophic hormone and cortisone in ocular disease. *Arch. Ophthalmol. Chicago*. **45**: 274-300.
3. STEFFENSEN, E. H., J. A. OLSON, R. R. MARGULIS, R. W. SMITH & E. L. WHITNEY. 1950. Experimental use of cortisone in inflammatory eye disease. *Am. J. Ophthalmol.* **33**: 1033-1040.
4. McDONALD, P. R., I. H. LEOPOLD, A. W. VOGEL & R. D. MULBERGER. 1953. Hydrocortisone (compound F) in ophthalmology. *Arch. Ophthalmol. Chicago*. **49**: 400-412.
5. LAVAL, J. 1953. Use of hydrocortisone (hydrocortone) acetate in ophthalmology. *Arch. Ophthalmol. Chicago*. **50**: 299-302.
6. STEFFENSEN, E. H., H. B. IVY & F. O. NAGLE. 1952. Topical compound F in the treatment of anterior-segment eye disease. *Am. J. Ophthalmol.* **35**: 933-934.
7. STEFFENSEN, E. H. 1952. Corticotropin, cortisone and hydrocortisone in treatment of ocular disease. *J. Am. Med. Assoc.* **150**: 1660-1664.
8. YASUNA, J. M., G. W. OJERS, W. C. FRAYER & H. G. SCHEIE. 1954. An experimental study of the effect of cortisone on the eye. *Am. J. Ophthalmol.* **37**: 923-931.
9. HANAN, R. & J. R. OVERMAN. 1953. Cortisone effect on antibody levels in rabbits simultaneously immunized with serum albumin and sheep cells. *Proc. Soc. Exptl. Biol. Med.* **84**: 420-423.
10. MIKULICICH, G. & Y. T. OESTER. 1953. Influence of adrenalectomy and cortisone on the anaphylactic heart reaction in rabbits. *J. Allergy*. **24**: 227-235.
11. PICKERING, G. W. 1952. Allergy and ACTH. *Brit. Med. J.* **1**: 1207-1210.
12. MENKIN, V. 1953. Significance of the accumulation of cortisone in an inflamed area. *Brit. J. Exptl. Path.* **34**: 412-419.
13. JONES, I. S. & K. MEYER. 1950. Inhibition of vascularization of rabbit cornea by local application of cortisone. *Proc. Soc. Exptl. Biol. Med.* **74**: 102-104.
14. CLAWSON, B. J. & S. T. NERENBERG. 1953. Effect of large doses of cortisone upon the ability of the reticuloendothelial cells to phagocytose streptococci. *J. Lab. Clin. Med.* **42**: 746-748.
15. GUYTON, J. S. Personal communication.
16. STEFFENSEN, E. H., A. J. WISHBOW, F. O. NAGLE, R. W. SMITH & E. L. WHITNEY. 1951. Topical cortisone in treatment of anterior-segment eye disease. *Am. J. Ophthalmol.* **34**: 345-356.

## C. NOSE

# CONTROLLED STUDIES IN THE TOPICAL APPLICATION OF HYDROCORTISONE IN VASOMOTOR RHINITIS

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Of the adrenal corticoids known to suppress inflammation, hydrocortisone or compound F is the most effective when given systemically,<sup>1, 2</sup> or when used locally on the skin,<sup>3</sup> in the eye,<sup>4</sup> or injected into the synovial joints.<sup>5</sup> Recent reports have also indicated that this hormone is a potent inhibitor of certain hypersensitive reactions,<sup>6, 7</sup> and that, when applied to the nasal mucus membranes, it will alleviate the symptoms of allergic rhinitis.<sup>8, 9</sup>

Individual variation in response to therapy is especially marked in the allergic patient whose severity of symptoms is influenced by various fluctuations in his physical, nutritional, or emotional state, and by certain environmental factors such as atmosphere and climate. Furthermore, the patient's own appraisal of his response to treatment is quite often not in accord with the physician's objective evaluation. Personal enthusiasm by the physician for his therapeutic plan may cause a biased interpretation of results.

Because of such factors, a controlled study was conducted to evaluate the results of topically applied hydrocortisone preparations in the treatment of patients with vasomotor rhinitis. The purpose of this paper is to report the method used and observations in this study.

### *Selection of Patients*

One hundred patients were chosen from clinical allergy practice after otolaryngological consultation. All had subjective and objective evidence of vasomotor rhinitis characterized by an edematous, pale, or sometimes inflamed nasal mucosa with or without polyp formation and superimposed infection. Subjective symptoms were usually a stuffy nose with rhinorrhea, or persistent postnasal drainage and, often, loss of sense of smell or taste. All patients were chosen at random without selection as to age, sex, or duration of symptoms. The specific etiology in each case was basically an inhalent allergy, involving various pollens, dust, or molds, and was confirmed by complete allergy evaluation. The patients studied comprised 42 males and 58 females. Ages ranged from 12 years to 64 years. Duration of symptoms varied from 3 months to 12 years.

No patient was included in this study who had physical or laboratory abnormalities that could not be attributed to the vasomotor rhinitis.

### *Method*

The "double blind" technique of clinical investigation was used in the study. Two preparations\* in identical plastic spray bottles labeled Preparation A and

\* All preparations were supplied through the courtesy of the Upjohn Pharmaceutical Co., Kalamazoo, Mich.



Preparation B were used. The exact contents were unknown to the patient and investigator throughout the study. One bottle contained hydrocortisone acetate (ester form) 15 mg./cc. (1.5 per cent) in a sodium chloride, sodium citrate, isotonic base. The other bottle contained the isotonic medium without the hydrocortisone.

All patients and medications were chosen at random so that no one group was receiving identical preparations at any one time. Each patient was given one or the other spray bottle and carefully instructed in the use in order to cover completely the nasal mucosa with the medication. If the nose was blocked, the material was to be used as nasal drops in the recumbent position. If the airway was patent, the spray technique was preferred. In each procedure, the dosage was approximately 0.30 ml., four times daily. Total dosage by this method was approximately 1.0 ml. to 1.5 ml. used in a 24 hour period. This program was continued for one month. At the end of that period each patient was interviewed, examined by a nose and throat consultant, and the other preparation given for a similar trial and comparison.

All patients studied had complete physical examination in addition to otolaryngological consultation. Routine laboratory studies including chest X ray, blood count, and serological tests for syphilis were evaluated.

Nasal smears were obtained by the method outlined by Hansel and examined microscopically at monthly intervals.<sup>10</sup> Cellular constituents of the exudate were recorded. Nasal polypi were removed from several patients, and the histological appearance of the tissue noted before and after the use of each preparation.

### *Results*

Subjective and objective findings were used to evaluate the results. Subjective indices were: (a) improvement in nasal stuffiness; (b) less nasal irritation, itching or sneezing; (c) improved sense of smell, or taste; and (d) reduced postnasal drainage. Objective means of classification were: (a) change in appearance of nasal mucous membrane; (b) reduction in size, or disappearance of nasal polypi; (c) lessening of nasal discharge; and (d) change in cellular exudate as noted by stained nasal smears.

Analysis of results showed subjective and/or objective improvement in 86 per cent of the patients receiving the hydrocortisone preparation, 64 per cent of these patients showing objective improvement. Subjective benefit was obtained by only 7 per cent of the patients receiving the placebo. No objective improvement occurred in this latter group.

Studies by the same method on 100 additional patients using a suspension of hydrocortisone acetate 5 mg. ml., of hydrocortisone free alcohol 5 mg./ml., and hydrocortisone free alcohol in solution 0.2 mg./ml. produced similar results. Less beneficial effect and a slight increase in severity of symptoms, however, was noted in the patients using the hydrocortisone free alcohol solution. Of this group, the most common complaints were of nasal irritation, a drying effect, or an unpleasant odor, or taste.

The complete analysis of results are tabulated in FIGURE 1 and TABLE 1.

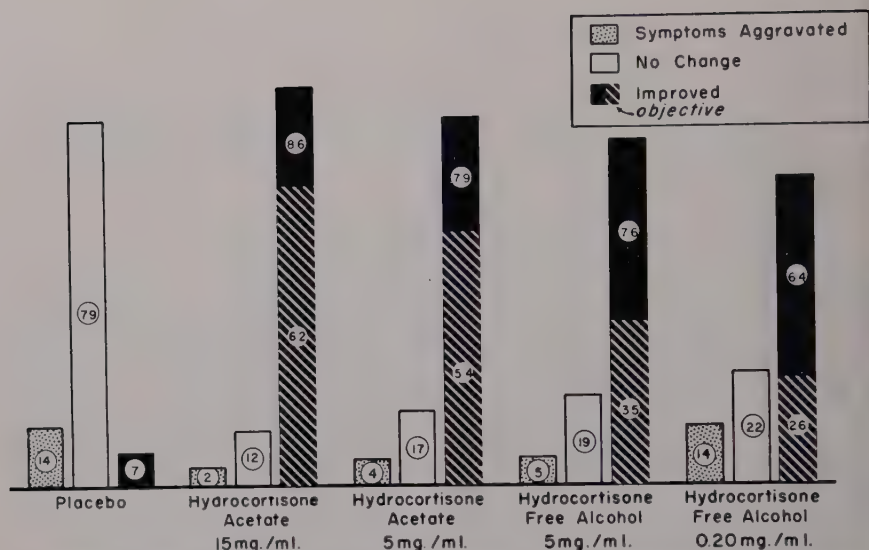


FIGURE 1. Comparison of the effects of different concentrations of hydrocortisone acetate and hydrocortisone free alcohol preparation in vasomotor rhinitis.

TABLE 1  
SUMMARY OF INDIVIDUAL GROUPS OF PATIENTS WITH VASOMOTOR RHINITIS AND THEIR RESPONSE TO LOCAL HYDROCORTISONE

Preparation	Study 1		Study 2		
	Placebo	Hydrocortisone acetate 15 mg./ml.	Hydrocortisone acetate 5 mg./ml.	Hydrocortisone free alcohol 5 mg./ml.	Hydrocortisone free alcohol solution 0.2 mg./ml.
Diagnosis:					
a) Vasomotor rhinitis.....	76	76	69	69	69
b) Vasomotor rhinitis with nasal polypi.....	24	24	31	31	31
Total patients.....	100	100	100	100	100
Results:					
Aggravation of symptoms					
a) Vasomotor rhinitis.....	4	2	2	4	11
b) Vasomotor rhinitis with polyp formation.....	10	0	2	1	3
No change:					
a) Vasomotor rhinitis.....	66	10	10	14	16
b) Vasomotor rhinitis with polyp formation.....	13	2	7	5	6
Improvement:					
Vasomotor rhinitis					
1) Objective.....	0	54	41	19	12
2) Subjective.....	6	10	16	32	30
Vasomotor rhinitis with polyp formation					
1) Objective.....	0	8	13	16	14
2) Subjective.....	1	14	9	9	8
Total patients.....	100	100	100	100	100

TABLE 2

EOSINOPHIL COUNTS OF 15 PATIENTS SHOWING NO REDUCTION IN CIRCULATING EOSINOPHILES DURING LOCAL THERAPY WITH HYDROCORTISONE ACETATE

Patient	Pretreatment eosinophile count	During treatment with hydrocortisone acetate 15 mg./ml.	
		4 hours	24 hours
1	143	130	78
2	40	56	104
3	265	240	214
4	84	80	76
5	120	122	115
6	38	75	60
7	198	175	216
8	134	78	150
9	80	96	78
10	202	165	122
11	40	74	58
12	32	58	45
13	238	188	194
14	80	70	66
15	122	115	125

### *Toxicity*

During the study, eosinophil counts were done on a random sampling of patients at 4 hours and 24 hours after intranasal use of the medication. No evidence of systemic absorption was noted by this method in the concentration used (TABLE 2). No significant adverse effect was evident in any patient during the period of observation.

### *Discussion*

Evidence available from this study indicates that hydrocortisone, when applied to the nasal mucous membranes, exerts a potent anti-inflammatory effect. There seems to be no significant statistical difference between the various preparations in suspension. The free alcohol solution shows a lesser beneficial effect, however, and a corresponding higher degree of irritating qualities, even though the pH 6.85 corresponded to the other preparations. The reason for this is not quite clear. It is postulated that the solution remained in physical contact for a shorter period of time than the suspension, or that the concentration of 0.20 mg./ml. was too low to be effective as an anti-inflammatory agent.

It is unlikely that the beneficial effect of the hormone is due to systemic absorption. Eosinophile counts, at regular intervals on a random sampling of patients, showed no evidence of absorption with the concentration used. Also the equal effectiveness of 5 mg./ml. preparations would indicate only local effect. If the total amount of the hormone used daily were completely absorbed, the 5 mg. to 8 mg. would hardly be physiologically significant.

Objective and subjective evaluation was used in the analysis of results of this study. It is recognized that subjective evaluation is highly individualized. When a double blind study is done, however, subjective evaluation becomes

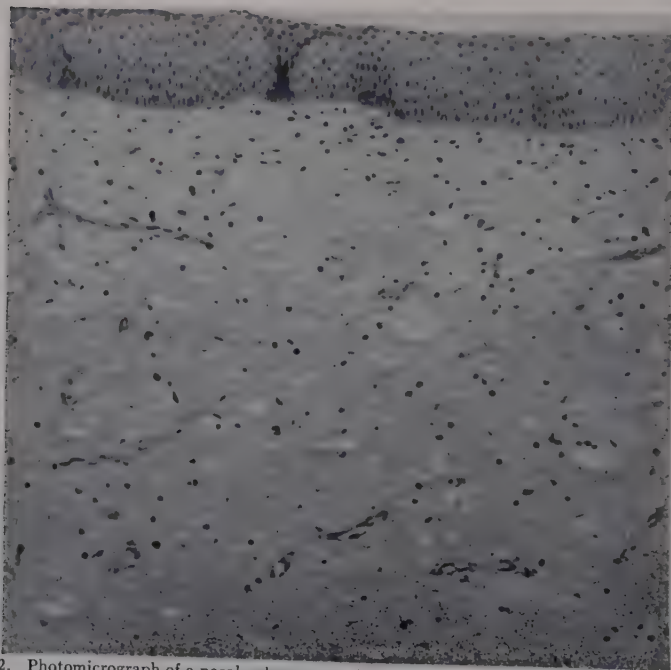
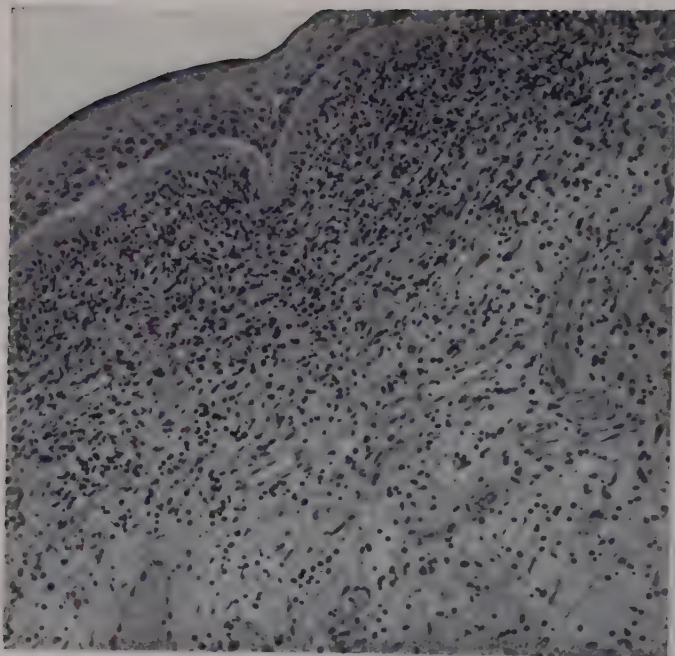


FIGURE 2. Photomicrograph of a nasal polyp removed after treatment with hydrocortisone acetate 15 mg./ml. in dosage of 0.3 ml. four times daily and continued for one month. There is marked histological variation in different areas of the polyp. These changes are not necessarily due to the topical application of hydrocortisone since other sections before therapy showed similar changes.



significant. This is especially true with the allergic patient whose objective findings may be meager, but whose symptoms are quite distressing.

Although there was a consistent reduction in size or disappearance of nasal polypi, there was no marked alteration of the nasal exudate, or change in histological appearance of the polypi after therapy. Nasal polypi removed after the use of each preparation were similar in appearance. Certain areas in each polyp, however, were relatively free of eosinophiles with other areas showing groups of eosinophiles and metaplasia of the epithelium. Examination of these areas by biopsy or by studying the histological appearance of the polyp in certain areas only would give an erroneous opinion of the effectiveness of the medication (FIGURE 2).

The effectiveness of therapy seems to depend upon the amount of hormone in contact with the inflamed mucous membrane. For this reason, it is important to obtain the patient's cooperation as to the exact use of the medication. If the airway was blocked, the medication was best used in drop form to cover the mucous membrane completely. If the airway was open, the spray technique was preferred. Written directions are best given the patient regarding the use of the medication.

No lasting benefit was obtained from the use of any of the preparations. Symptoms returned in all patients 10 to 14 days after discontinuing therapy. Although hydrocortisone is a potent anti-inflammatory substance and an effective adjunct in the management of patients with vasomotor rhinitis, it does not eliminate the need for avoidance of offending allergens or hyposensitization therapy.

### *Summary*

Controlled studies in the topical application of various preparations of hydrocortisone in several concentrations in 200 patients with vasomotor rhinitis are presented. Results show marked anti-inflammatory effect and symptomatic improvement in 76 per cent to 86 per cent of the patients using hydrocortisone suspensions. Sixty-four per cent improved using hydrocortisone free alcohol solution in concentration of 0.2 mg./ml.

No significant systemic absorption, adverse effects, change in nasal exudate, or alteration of the histological appearance of nasal polypi were noted after therapy.

Although topically applied hydrocortisone is of significant symptomatic benefit in the treatment of patients with vasomotor rhinitis, it does not eliminate the necessity for proper allergic management.

### *References*

1. COCHRAN, G. C., J. P. JAHN, N. FOREMAN, L. W. KINSELL & F. OLSON. 1953. Evaluation of adrenal steroids administered intravenously, intramuscularly and orally. *J. Clin. Endocrinol. Metabolism*. **13**: 993-996.
2. CONN, J. W. *et al.* 1951. Metabolic effects in management of orally and parenterally administered compound F (17-Hydroxycorticosterone) and compound F acetate. *J. Lab. Clin. Med.* **38**: 799.
3. SULZBERGER, M. B., V. H. WITTEN & C. C. SMITH. 1953. Hydrocortisone (compound F) acetate in dermatological therapy. *J. Am. Med. Assoc.* **151**: 468-472.
4. STEFFENSEN, E. H. 1952. Corticotropin, cortisone, and hydrocortisone in treatment of ocular disease. *J. Am. Med. Assoc.* **150**: 1660-1664.

5. JESSAR, R. A., M. A. GANZELL & C. RAGAN. 1953. The action of hydrocortisone in synovial inflammation. *J. Clin. Invest.* **32**: 480-482.
6. GOLDMAN, L., R. H. PRESTON, E. ROCKWELL & J. BASKET. 1952. Inhibition of tuberculin reaction by local injection of compound F. *J. Am. Med. Assoc.* **150**: 30-31.
7. TRAYNOR, M. V., JR. *et al.* 1954. Hydrocortisone treatment of pollinosis. *Ann. Allergy.* **12**: 263-265.
8. SILCOX, L. E. 1954. The intranasal use of hydrocortisone alcohol. Scientific Exhibition Section on Otolaryngology. San Francisco Session Am. Med. Assoc., San Francisco Calif.
9. SMITH, T. T. 1954. Local use of hydrocortisone acetate in the nose. *Arch. Otolaryngol.* **60**: 24-36.
10. HANSEL, F. K. 1934. Observation on the cytology of the secretion in allergy of the nose and paranasal sinuses. *J. Allergy.* **5**: 357-366.
11. DILL, J. L. & D. S. BOLSTAD. 1952. Further observations on the local use of cortisone in the nose in allergic rhinitis. *J. Am. Acad. Ophthalmol. Otolaryngol.* **56**: 214-219.

## Part V. Halogenated Analogs of Hydrocortisone

### A. FLUOROHYDROCORTISONE

#### BIOLOGICAL EFFECTS OF 9- $\alpha$ -FLUOROHYDROCORTISONE AND RELATED HALOGENATED STEROIDS IN ANIMALS

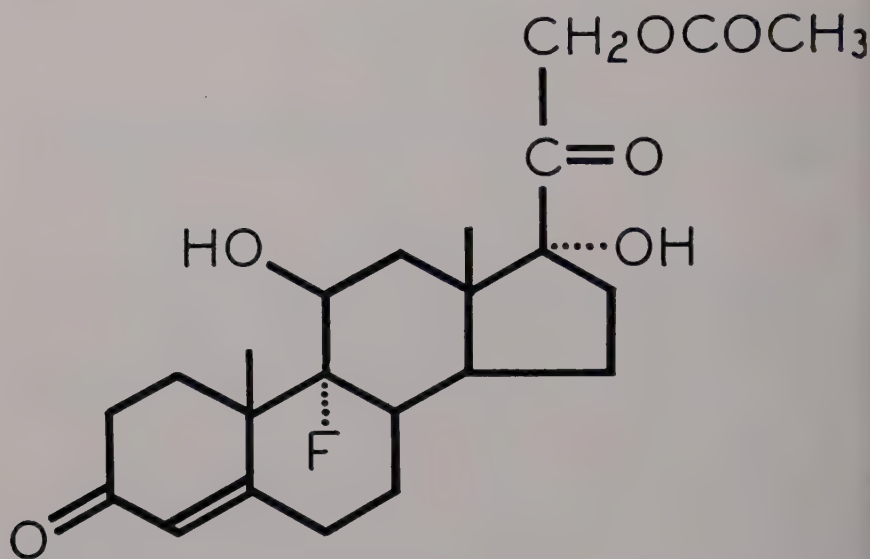
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Attempts to enhance or modify the activities of the cortical hormones by altering their chemical structure have been the subject of numerous investigations ever since the discovery by Hench *et al.*<sup>1</sup> of the dramatic effects of cortisone in rheumatoid arthritis. The disappointing record of achievement in this direction<sup>2</sup> prior to the discovery of the 9 $\alpha$ -halo compounds attests to the exacting structural requirement for corticoid activity.

The discovery of the unusual properties of the 9 $\alpha$ -halogenated steroids<sup>3</sup> was made in the course of an investigation of the synthesis of hydrocortisone from 11-epihydrocortisone, a biologically inactive, readily available stereoisomer of the former. Two intermediates in this synthesis, 9 $\alpha$ -bromo- and 9 $\alpha$ -iodohydrocortisone acetate, when subjected to the rat liver glycogen assay of Pabst, Shepard, and Kuizenga,<sup>4</sup> proved to possess activities of about one third and one tenth that of cortisone acetate respectively.<sup>3, 5</sup> It appeared logical to speculate that substitution at the 9-position by the lighter halogens, chlorine and fluorine, might produce compounds of greater activity than that of the bromo compound. This was borne out in the most unexpected manner. 9 $\alpha$ -Chlorohydrocortisone acetate<sup>3</sup> and 9 $\alpha$ -fluorohydrocortisone acetate,<sup>6</sup> as well as the corresponding cortisone derivatives, proved to be even more active than the halogen-free substances from which they were derived. In fact, 9 $\alpha$ -fluorohydrocortisone acetate (FIGURE 1), the most active member of this group, was found to be 11 times as active as cortisone acetate.<sup>6</sup> The results for these and other 9 $\alpha$ -substituted derivatives of cortisone and hydrocortisone in the liver glycogen assay are listed in the first column of TABLE 1. It is seen that the activity increases with decreasing size of the halogen atom. Substitution of a hydroxyl group for a halogen atom resulted in a compound of low activity. Methoxyl and ethoxyl substitution led to far-reaching, if not complete, inactivation. It was also found that the activity of these steroids in the liver glycogen test remained the same whether they were administered by the oral or subcutaneous route. Other manifestations of glucocorticoid activity, such as effects on thymus weight<sup>5</sup> and on the excretion of a water load,<sup>5</sup> paralleled the results obtained in the liver glycogen assay.

When these steroids were assayed for their ability to maintain the life of adrenalectomized rats by the growth-survival technique of Gaunt *et al.*,<sup>7</sup> Borman, Singer, and Numerof<sup>8</sup> found that the bromo- and chloro- derivatives of both cortisone and hydrocortisone acetate were not only more active than cortisone and hydrocortisone acetate but considerably exceeded the activity of desoxycorticosterone in their effects on both growth and survival time. This



# 9 $\alpha$ -FLUOROHYDROCORTISONE ACETATE

FIGURE 1

TABLE 1  
ADRENOCORTICOID ACTIVITY OF 9-HALO-HYDROCORTISONES AND CORTISONES IN THE  
ADRENALECTOMIZED RAT

Steroid	Liver glycogen	Water diuresis	Thymus involution	Survival	Na <sup>22</sup> retention
	Cortisone acetate = 1			DCA = 1	
9 $\alpha$ -Iodo F acetate..	0.1	—	—	—	—
9 $\alpha$ -Bromo F acetate	0.28 (0.24-0.32)	0.5	0.1	2-5	<1
9 $\alpha$ -Bromo E acetate	0.54 (0.47-0.62)	0.5	0.1	5-10	0.75 (0.36-1.55)
9 $\alpha$ -Chloro F acetate	4.7 (2.7-8.2)	5.0	2.0	10-20	10.8 (6.8-17.1)
9 $\alpha$ -Chloro E acetate	3.7 (2.6-5.2)	2.0	2.0	10-20	7.00 (4.14-11.82)
9 $\alpha$ -Fluoro F acetate	10.7 (8.4-13.6)	10.0	4.0	<1	0.68 (0.44-1.05)
9 $\alpha$ -Fluoro E acetate	9.0 (6.3-12.8)	—	—	3-5	2.78 (1.49-5.21)
9 $\alpha$ -Hydroxy F acetate.....	0.2	—	—	—	<0.1
9 $\alpha$ -Methoxy F acetate.....	<0.2	—	—	—	<0.1
9 $\alpha$ -Ethoxy F acetate.....	<0.2	—	—	—	<0.2

The figures in parentheses represent the 95 per cent confidence intervals calculated by the method of C. I. Bliss (*The Statistics of Bioassay*, New York, Academic Press, 1952).



TABLE 2

INFLUENCE OF THE SIDE CHAIN UPON ADRENOCORTICOID ACTIVITY OF 9 $\alpha$ -HALO STEROIDS IN THE ADRENALECTOMIZED RAT

Steroid						
	Liver glycogen Cortisone acetate = 1	Sodium retention DCA = 1	Liver glycogen Cortisone acetate = 1	Sodium retention DCA = 1	Liver glycogen Cortisone acetate = 1	Sodium retention DCA = 1
X = Br, Y = OH	—	<0.02	—	<0.1	—	—
X = Br, Y = O	—	—	0.07 (0.03-0.15)	—	—	—
X = Cl, Y = OH	0.35 (0.27-0.44)	<0.02	1.15 (0.67-1.96)	0.87 (0.48-1.57)	0.06 (0.04-0.08)	<0.02
X = Cl, Y = O	—	—	—	1.1 (0.5-2.5)	—	—
X = F, Y = OH	0.85 (0.69-1.04)	1.14 (0.53-2.48)	4.58 (3.33-6.30)	26.2 (10.9-66.0)	1.88 (1.47-2.40)	<0.1
X = F, Y = O	1.10 (0.87-1.38)	2.84 (1.26-6.36)	5.90 (4.83-7.21)	20.8 (9.6-44.7)	0.81 (0.67-0.98)	<0.1

The figures in parentheses represent the 95 per cent confidence intervals calculated by the method of C. I. Bliss (*The Statistics of Bioassay*, New York, Academic Press, 1952).

TABLE 3

ANTI-INFLAMMATORY ACTIVITY OF SOME 9 $\alpha$ -HALOSTEROIDS

Steroid	Anti-inflammatory activity	Liver glycogen assay
	Cortisone acetate = 1	
9 $\alpha$ -Fluorohydrocortisone acetate.....	13.19 (8.70-20.00)	10.7 (8.4-13.6)
9 $\alpha$ -Chlorohydrocortisone acetate.....	2.76 (1.72-4.40)	4.7 (2.7-8.2)
9 $\alpha$ -Fluorocorticosterone acetate.....	2.69 (1.82-3.96)	4.6 (3.3-6.3)
9 $\alpha$ -Fluoro-11 $\beta$ ,17 $\alpha$ -dihydroxyprogesterone.....	0.37 (0.16-0.85)	1.9 (1.5-2.4)
9 $\alpha$ -Fluoro-11 $\beta$ -hydroxyprogesterone....	<0.1	0.85 (0.7-1.0)

finding prompted the determination of the sodium-retaining activity of these compounds with the results shown in the last column of TABLE 4. As had been anticipated from the results in the growth-survival test, the chloro-derivatives exceeded all others in this group in their effects on sodium retention. On the other hand, the relatively high life-maintenance activity of the bromo com-

TABLE 4

EFFECT OF SOME 9 $\alpha$ -HALOSTEROIDS ON SODIUM RETENTION AND CIRCULATING EOSINOPHILES  
IN ADRENALECTOMIZED DOGS  
(Liddle, Pechet, and Bartter)

Steroid	Sodium retention DCA = 1	Eosinopenia F acetate = 1
9 $\alpha$ -Chloro-F acetate.....	3.3 (1.9-5.2)	8 (5-13)
9 $\alpha$ -Chloro-E acetate.....	2.1 (1.2-3.8)	—
9 $\alpha$ -Fluoro-F acetate.....	4.7 (2.4-9.2)	20 (11-36)

TABLE 5

LIFE MAINTENANCE ACTIVITY OF SOME 9 $\alpha$ -HALOSTEROIDS IN ADRENALECTOMIZED DOGS  
(Swingle, Baker, Eisler, Le Brie, and Brannick)

Steroid	Minimum maintenance dose/dog/day $\mu$ g.	Potency DOC = 1
9 $\alpha$ -Chloro F acetate.....	55-100	2.27
9 $\alpha$ -Fluoro F acetate.....	28	4.5-9.1
9 $\alpha$ -Fluorocorticosterone acetate.....	7-14	18.2
9 $\alpha$ -Fluoro-11 $\beta$ -hydroxyprogesterone.....	150	0.83-1.7
9 $\alpha$ -Fluoro-11 $\beta$ ,17 $\alpha$ -dihydroxyprogesterone....	300	0.42-0.83
Aldosterone.....	6-12	20
Hydrocortisone.....	5000	0.025-0.050
Cortisone.....	5000	0.025-0.050

pounds was not accompanied by equally pronounced effects upon sodium retention. The trend of increasing mineralocorticoid activity established for the bromo- and chloro- compounds was not maintained with the fluoro- compounds. 9 $\alpha$ -Fluorohydrocortisone acetate was found to be one of the least active members of this group. Its efficacy in retaining sodium was only about equal to that of desoxycorticosterone acetate, and its effects upon growth and survival time even less pronounced than those of DCA. This relatively low degree of activity of fluorohydrocortisone acetate in these tests seems to be peculiar to the rat, since both in the dog and in man, as will be pointed out later, this compound is extremely active and surpasses the chloro- compounds in both sodium retention and life maintenance activity (*cf.* TABLES 4 and 5). Fluorocortisone acetate was definitely more active than the hydrocortisone derivative in both tests.

The growth-survival technique used in these experiments employs a single injection immediately after adrenalectomy. Such an experimental design appraises not only the activity of the steroids under test but also the longevity of their action. Leatham and Wolf<sup>9</sup> have determined the life-maintaining action of chlorohydrocortisone acetate in the adrenalectomized rat by giving daily injections. In their test, chloro-F acetate showed activity only about equal to that of desoxycorticosterone acetate whereas, by the single injection technique, this compound exhibited about 11 times the activity of DCA. This provides an indication of the longevity of action of chloro-F acetate as compared to DCA.

It appeared of great interest to ascertain what influence variations in the

side chain might have upon the adrenocorticoid activity of  $9\alpha$ -halogenated derivatives. For this purpose we have prepared the  $9\alpha$ -halo derivatives (halogen:chlorine, bromine and fluorine) of  $11\beta$ -hydroxyprogesterone,  $11\beta$ ,  $17\alpha$ -dihydroxyprogesterone and corticosterone acetate and of the corresponding 11-ketones, and assayed them for their liver glycogen and sodium retention activity in the adrenalectomized rat.<sup>10</sup> The results are listed in TABLE 2. It is apparent, even on cursory examination of this table, that both glucocorticoid and mineralocorticoid activities increase with decreasing atomic weight of the halogen atom. With the exception of the unusually low mineralocorticoid activities encountered with the fluoro- derivatives, this was also true for the  $9$ -halohydrocortisones and cortisones. No significant differences were noted between the activities of the  $11\beta$ -hydroxy and 11-keto derivatives. The most striking effects were again exhibited by the fluoro compounds. Thus,  $9\alpha$ -fluoro- $11\beta$ -hydroxyprogesterone and  $9\alpha$ -fluoro-11-ketoprogesterone, although lacking both the 17- and 21-hydroxyl groups, approximately equaled cortisone acetate in their glucocorticoid and desoxycorticosterone acetate in their mineralocorticoid action.  $9\alpha$ -Fluorocorticosterone acetate and  $9\alpha$ -fluorodehydrocorticosterone acetate were not only highly active in the liver glycogen test (4.6 and 5.9 times cortisone acetate, respectively) but also represented the most potent mineralocorticoids so far encountered by us. These two steroids possess between 20 and 30 times the activity of DCA, which aligns them with aldosterone as the most powerful sodium retaining substances known. Aldosterone has been reported<sup>11, 12</sup> to possess 20 to 30 times the activity of desoxycorticosterone.

The anti-inflammatory activity of a number of  $9\alpha$ -halogenated steroids in adrenalectomized rats has been determined by Singer and Borman.<sup>13</sup> The method used by these authors is a modification of the cotton pellet implant technique described by Meyer, Stucki, and Aulsebrook,<sup>14</sup> and consists of subcutaneously implanting 5 to 7 mg. cotton pellets in the upper dorsal area, followed by four daily injections of an aqueous suspension of the steroid under test. Ninety-six hours after implantation, the pellets are carefully dissected from the animals, along with the accumulated granulation tissue. The wet and, preferably, the dry weights of the pellets serve as indicators of the inflammatory response. Linear dose-response relationships have been established for most of the steroids tested. The data obtained are summarized in TABLE 3. For purposes of comparison, the liver glycogen depository activities of the steroids are listed side by side with the potencies established in the anti-inflammatory test. With the exception of the two substances at the bottom of the table, there is good correlation between the values obtained in the two tests. The discrepancies observed with the latter two substances may be due to their solubility characteristics or may reflect the different ways in which the steroids are administered in the two tests. In the liver-glycogen assay, the compounds are administered in 25 per cent Tween solution, while the anti-inflammatory test employs aqueous suspensions of the steroids.

*Activity of  $9\alpha$ -halosteroids in adrenalectomized dogs.* Liddle, Pechet, and Bartter<sup>15</sup> have determined the sodium-retaining activity of  $9\alpha$ -fluorohydrocortisone acetate,  $9\alpha$ -chlorohydrocortisone acetate, and  $9\alpha$ -chlorocortisone

acetate in adrenalectomized dogs. At dose levels of 25 to 100  $\mu\text{g.}$ , these steroids were found to induce sodium retention and potassium loss in direct proportion to the dosage administered. Conversely, when given in doses larger than 500  $\mu\text{g.}$ , these same steroids frequently induced sodium loss. These authors also observed that such high doses regularly produced increases in the glomerular filtration rate (GFR) and explain the divergent effects dependent on dosage by assuming that the halosteroids, like DCA, cause an increase in the tubular reabsorption of sodium at all dose levels. At the higher levels, however, the increase in the GFR may present more sodium to the tubules than can be reabsorbed, leading to the observed net loss of sodium. This same hypothesis may also serve to explain the sodium losses after high doses of cortisone and hydrocortisone, since such losses were likewise accompanied by increases in the GFR.

At the lower dose levels, where sodium retention followed a linear dose-response relationship, potencies relative to DCA could be calculated for each steroid. These values are listed in TABLE 4. The most striking feature in these results is the fact that, in the dog, fluorohydrocortisone acetate is more active than chlorohydrocortisone acetate, whereas the reverse was found to be true in the rat (*cf.* TABLE 1).

As an index of glucocorticoid activity, TABLE 4 also lists the eosinopenic potencies of 9 $\alpha$ -fluoro- and 9 $\alpha$ -chlorohydrocortisone acetates relative to hydrocortisone acetate in the adrenalectomized dog.

Swingle *et al.*<sup>16, 17</sup> have tested a number of 9 $\alpha$ -halogenated steroids for their ability to maintain adrenalectomized dogs and compared the results with those obtained in similar tests using aldosterone, DOC, cortisone, and hydrocortisone. The steroids were dissolved in 95 per cent alcohol and injected subcutaneously as 10 to 15 per cent alcoholic solutions twice daily in divided doses. The daily intake of Na and K per dog was 1.47 g. and 0.94 g., respectively. Dosage was reduced by 50 per cent every 10 days and the following determinations were made: serum Na, Cl, and K, blood pressure, blood urea nitrogen, blood glucose, hematocrit, hemoglobin and red blood cells. The minimum maintenance dose was considered to be the daily amount required to maintain the animals symptom-free, and to keep the blood constituents and blood pressure changed little if at all from the normal or starting values. The minimum maintenance doses and the potencies relative to desoxycorticosterone are listed in TABLE 5. The data for 9 $\alpha$ -fluorohydrocortisone acetate and 9 $\alpha$ -chlorohydrocortisone acetate parallel those reported by Liddle, Pechet, and Bartter in the sodium retention assay (*cf.* TABLE 4). The most active compound tested was 9 $\alpha$ -fluorocorticosterone acetate, which proved to be no less potent than aldosterone. It will be remembered that this compound also simulated aldosterone in its ability to induce sodium retention in the adrenalectomized rat (TABLE 2). Of the two remaining fluoro- derivatives tested, 9 $\alpha$ -fluoro-11 $\beta$ -hydroxyprogesterone was about equally active in maintaining the life of adrenalectomized dogs as in inducing sodium retention in the rat, while 9 $\alpha$ -fluoro-11 $\beta$ ,17 $\alpha$ -dihydroxyprogesterone was considerably more active in the dog than it was in the rat.

Administering doses of 3 mg./kg./day of 9 $\alpha$ -fluorohydrocortisone perorally to two adrenalectomized dogs, Swingle *et al.*<sup>16</sup> observed profound effects upon



water intake and output. The maximum urine volume obtained for any 24-hour period was 7125 ml. for the one, and 4140 ml. for the other dog studied, which compares with a volume of 250 ml./day prior to treatment with the steroid. The animals also developed marked edema of the hind limbs and scrotum, which disappeared after discontinuing steroid administration.

It appears profitable, before closing, to assemble in a single table (TABLE 6) all the data obtained with the four  $9\alpha$ -fluoro- $11\beta$ -hydroxy derivatives differing in their structures solely by the degree and position of hydroxylation of the side chain and to evaluate the influence of this latter feature on the activities observed. Substitution of a hydroxyl group for a hydrogen atom in the  $17\alpha$ -position effects in all cases studied increases in both glucocorticoid and anti-inflammatory action, and a decrease in mineralocorticoid action. Substitution of an acetoxy group in the 21-position causes considerable enhancement of both glucocorticoid and mineralocorticoid activity. Hydroxylation in both the 17- and 21-positions produces in all cases the qualitative effects predictable on

TABLE 6  
DEGREE OF HYDROXYLATION OF THE SIDE CHAIN AND CORTICOID ACTIVITY OF  
 $9\alpha$ -FLUORO- $11\beta$ -HYDROXY STEROIDS

Side chain	Liver glycogen rat	Anti- inflammatory test, rat	Sodium retention rat	Life maintenance dog
	Cortisone acetate = 1		DCA = 1	
$\begin{array}{c} \text{CH}_3 \\   \\ \text{C}=\text{O} \\   \\ \text{CH} \\ / \quad \backslash \end{array}$	0.85	<0.1	1.1	0.83-1.7
$\begin{array}{c} \text{CH}_3 \\   \\ \text{C}=\text{O} \\   \\ \text{CH} \cdots \text{OH} \\ / \quad \backslash \end{array}$	1.9	0.37	<0.1	0.42-0.83
$\begin{array}{c} \text{CH}_2\text{OAc} \\   \\ \text{C}=\text{O} \\   \\ \text{CH} \\ / \quad \backslash \end{array}$	4.7	2.7	26.2	18.2
$\begin{array}{c} \text{CH}_2\text{OAc} \\   \\ \text{C}=\text{O} \\   \\ \text{CH} \cdots \text{OH} \\ / \quad \backslash \end{array}$	10.7	13.2	0.68	4.5-9.1

the basis of the results obtained with the singly hydroxylated products. In the case of the rat liver glycogen assay and the life maintenance test in the dog, there is quantitative agreement between the predicted and found potency values.

### Summary

9 $\alpha$ -fluorohydrocortisone, the main subject of this discussion, is a member of a group of physiologically active compounds, several of which are considerably more potent than the naturally-occurring cortical hormones.

Structurally, this group of compounds consists of all possible permutations derivable from 9 $\alpha$ -fluorohydrocortisone by replacing the substituents in the following positions as indicated: 9 $\alpha$ : F by Cl and Br; 11:  $\beta$ -OH by = O; 17 $\alpha$ : OH by H; and 21: OH by H.

The members of this group of steroids are discussed with regard to their effects on (1) deposition of liver glycogen; (2) water diuresis; (3) thymus involution; and (4) granuloma formation in adrenalectomized rats, as well as (5) sodium retention; and (6) life maintenance in adrenalectomized dogs and rats.

One of the most remarkable features of these compounds is the fact that many of them combine a high degree of mineralocorticoid (DOC-like) activity with high glucocorticoid (cortisone-like) action. Thus, the most active glucocorticoid, 9 $\alpha$ -fluorohydrocortisone acetate is 11 times more active than cortisone acetate in restoring liver glycogen deposition in the adrenalectomized rat and, at the same time, 4.5 to 9 times more active than DOC in maintaining the life of adrenalectomized dogs. The most active mineralocorticoid is 9 $\alpha$ -fluorocorticosterone, which is 18 times more active than DOC (or equal to aldosterone) in the latter, and 4.6 times more potent than cortisone acetate in the former test.

The following generalizations concerning the relationship between chemical structure and biological activity can be made:

(1) Lowering of the atomic weight of the halogen atom leads in all the cases examined to increased glucocorticoid activity in both rats and dogs. Mineralocorticoid-activity is always increased in dogs and, with few exceptions, also in rats.

(2) In the fluorinated derivatives, substitution of a hydroxyl group for a hydrogen atom in position 21 leads to a fivefold increase in glucocorticoid and to a tenfold to twentyfold increase in mineralocorticoid activity, while similar substitution in the 17 $\alpha$ -position causes only a twofold to threefold increase in glucocorticoid, and a decrease in mineralocorticoid action.

*Acknowledgments.* The author wishes to make it clear that, being a chemist, he has contributed to the data reviewed in this paper only in an indirect manner. He feels indeed deeply appreciative of the privilege of presenting this report. Grateful acknowledgment is made to Professor W. W. Swingle of the Biological Laboratory, Princeton University, and to Doctor A. Borman and Doctor F. M. Singer of our laboratories for kindly permitting the use of as-yet-unpublished data. To all those whose data are reported in this paper, the

author wishes to express his sincere appreciation for the spontaneous interest and enthusiasm they have shown for working with the compounds he and his collaborators have had the good luck to prepare.

### References

1. HENCH, P. S., E. C. KENDALL, C. H. SLOCUMB & H. F. PÓLLEY. 1949. Proc. Staff Meetings Mayo Clin. **24**: 181.
2. TISHLER, M. 1952. Record Chem. Progress (Kresge-Hooker Sci. Lib.) **13**: 161.
3. FRIED, J. & E. F. SABO. 1953. J. Am. Chem. Soc. **75**: 2273.
4. PABST, M. L., R. SHEPPARD & M. H. KUIZENGA. 1947. Endocrinology. **41**: 55.
5. BORMAN, A. & F. M. SINGER. 1954. Federation Proc. **13**: 185.
6. FRIED, J. & E. F. SABO. 1954. J. Am. Chem. Soc. **76**: 1455.
7. GAUNT, R., J. H. LEATHEM, C. HOWELL & N. ANTONCHAK. 1952. Endocrinology. **50**: 521.
8. BORMAN, A., F. M. SINGER & P. NUMEROF. 1954. Proc. Soc. Exptl. Biol. Med. **86**: 570.
9. LEATHEM, J. H. & R. C. WOLF. 1954. Proc. Soc. Exptl. Biol. Med. **86**: 724.
10. FRIED, J., J. E. HERZ, E. F. SABO, A. BORMAN, F. M. SINGER & P. NUMEROF. 1955. J. Am. Chem. Soc. **77**: 1068.
11. DESAULLES, P., J. TRIPOD & W. SCHULER. 1953. Schweiz. Med. Wochschr. **83**: 1088.
12. AXELROD, J., J. E. CATES, B. B. JOHNSON & J. A. LUETSCHER, JR. 1954. Endocrinology. **55**: 568.
13. SINGER, F. M. & A. BORMAN. 1955. Federation Proc. **14**: 281.
14. MEYER, R. K., J. C. STUCKI & K. A. AULSEBROOK. 1953. Proc. Soc. Exptl. Biol. Med. **84**: 624.
15. LIDDLE, G. W., M. M. PECHET & F. L. BARTTER. 1954. Science. **120**: 496.
16. SWINGLE, W. W., C. BAKER, M. EISLER, S. J. LE BRIE & L. J. BRANNICK. 1955. Proc. Soc. Exptl. Biol. Med. **88**: 193.
17. SWINGLE, W. W., C. BAKER, M. EISLER, S. J. LE BRIE & L. J. BRANNICK. 1955. Federation Proc. **14**: 150.

## BIOLOGICAL EFFECTS OF FLUORINATED DERIVATIVES OF HYDROCORTISONE AND PROGESTERONE IN MAN\*

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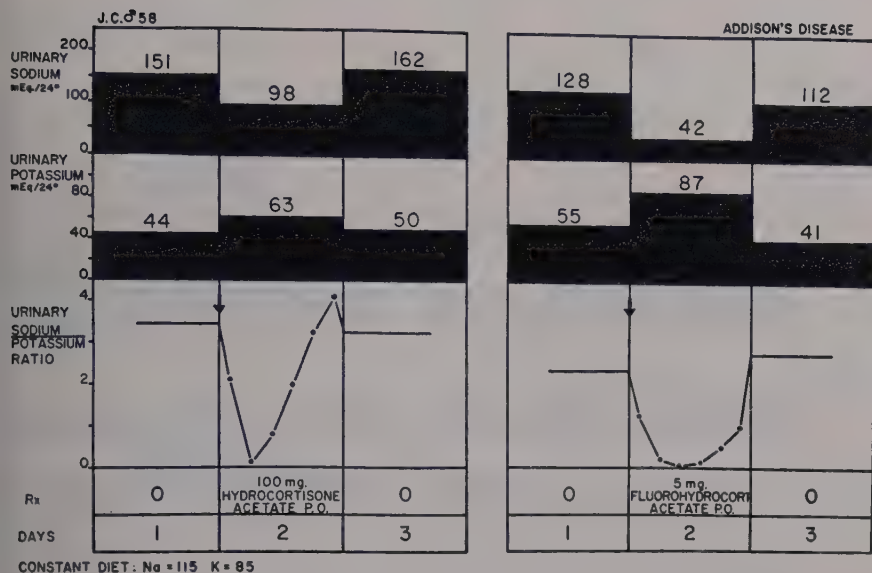
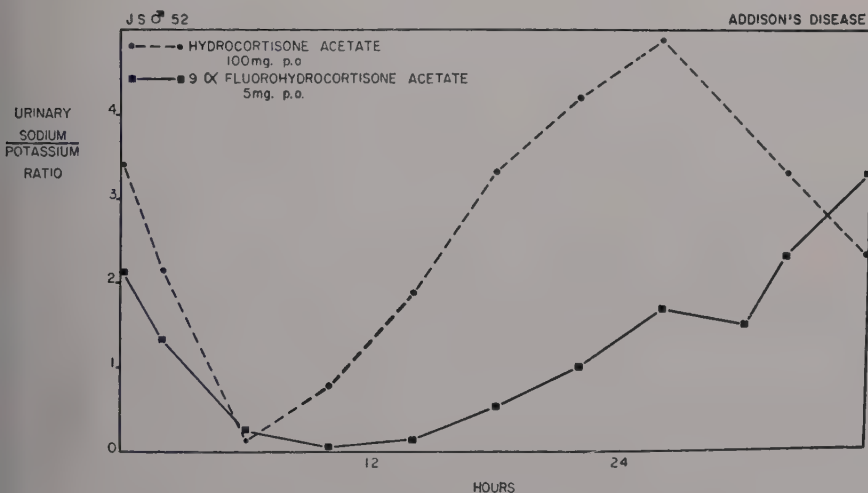
The biological effects of halogenated steroids in animals have been reviewed in this monograph by Doctor Fried and his collaborators.<sup>1-3</sup> The high degree of potency which these compounds demonstrated in the animal assays clearly indicated the desirability of a clinical evaluation of their biological activity in man.<sup>4, 5</sup> It has been possible to test a number of these derivatives of hydrocortisone and to compare their metabolic effects with those of the nonfluorinated parent compounds. Among them, 9-alpha-fluorohydrocortisone appeared to be the most promising from a clinical point of view and was made available in amounts sufficient for adequate clinical testing. The results obtained with other derivatives are to be considered, at present, as preliminary. These studies have been carried out on the Metabolic Ward of the Peter Bent Brigham Hospital, Boston, Mass.

The high degree of activity of 9-alpha-fluorohydrocortisone acetate is illustrated in FIGURE 1. The effects of 100 mg. of hydrocortisone acetate and of 5 mg. of fluorohydrocortisone acetate on urinary electrolyte excretion were compared in an Addisonian patient maintained on a constant diet. The two compounds were administered as a single dose by mouth. It is apparent that both sodium retention and potassium diuresis were considerably more marked with the fluorinated compound, indicating at least a twentyfold increase in sodium-retaining activity. Furthermore, when the urinary sodium-to-potassium ratio was followed (FIGURE 2), it appeared that both hydrocortisone and fluorohydrocortisone produced a marked and rapid decrease in this ratio, but that the effects of 5 mg. of fluorohydrocortisone were considerably prolonged as compared to those of 100 mg. of hydrocortisone.

The marked effects of 9-alpha-fluorohydrocortisone on organic metabolism are illustrated in FIGURE 3. A patient with Addison's disease maintained on a constant diet was given 25 mg. of the compound as an eight-hour intravenous infusion. The steroid was dissolved in 10 ml. of absolute ethanol and diluted in 500 ml. of saline containing 5 grams of albumin. The metabolic effects obtained were compared with those of a control infusion of saline with alcohol and albumin. A maximal and prolonged eosinopenia, a marked increase in urinary glucose excretion, and a definite rise in the urinary excretion of nitrogen, uric acid, and potassium were observed with fluorohydrocortisone. Urinary glucose was measured by a specific, enzymatic method based on the oxidation of glucose by glucose oxidase.<sup>6</sup> Employing this method with a patient main-

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FIGURE 1. Comparative effects of hydrocortisone and 9- $\alpha$ -fluorohydrocortisone.FIGURE 2. Effects of hydrocortisone and 9- $\alpha$ -fluorohydrocortisone.

tained on a constant diet, variations in daily glucose excretion under controlled circumstances were very small.

Quantitative comparative studies, using different dosages and different modes of administration,<sup>5</sup> have suggested that the substitution of a fluorine atom for hydrogen in position 9- $\alpha$  leads, in man, to a potentiation of the glucocorticoid activity of hydrocortisone by a factor of approximately 20. The

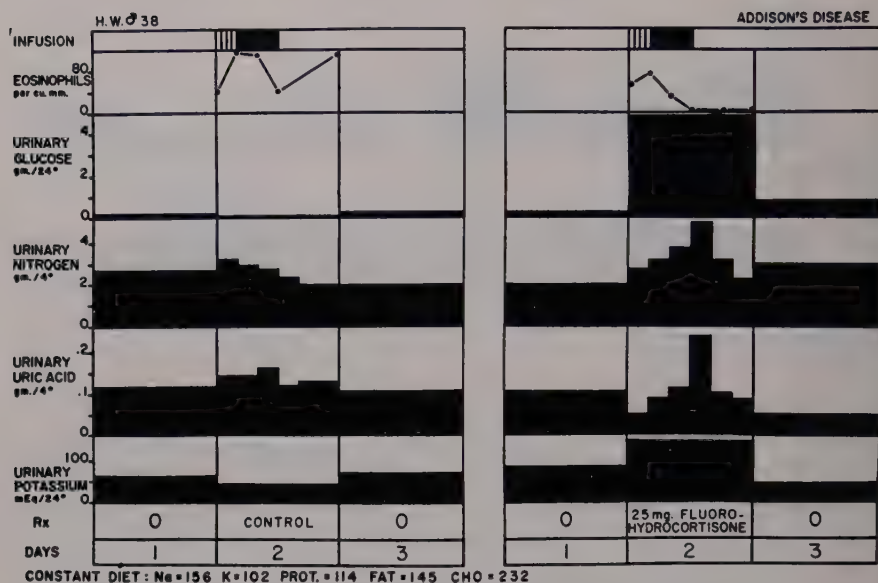


FIGURE 3. Effects of 9-alpha-fluorohydrocortisone on organic metabolism.

electrolyte effects, however, appear to be potentiated by an appreciably higher factor. Indeed, it would appear that the mineralocorticoid potency of 9-alpha-fluorohydrocortisone approaches or exceeds that of aldosterone.<sup>7</sup> This differential potentiation of the two principal biological activities of the steroid is of both theoretical and practical interest and is further illustrated in FIGURE 4. The effect of 100 mg. of hydrocortisone (free alcohol) and of 2 mg. of fluorohydrocortisone (free alcohol) were compared in a normal subject maintained

Time	Sodium mEq./2 hr.		Potassium mEq./2 hr.		Eosinophils per cu. mm.		Glucose mg. %		Uric Acid mg. %	
	Fluoro-F	Comp. F	Fluoro-F	Comp. F	Fluoro-F	Comp. F	Fluoro-F	Comp. F	Fluoro-F	Comp. F
7 a.m.					216	200	69	66		
	61	77	12	11					120	139
9 a.m.					156	106	71	79		
	26	42	19	11					110	113
11 a.m.					144	38	73	82		
	14	29	19	19					103	140
1 p.m.					119	3	79	99		
	16	23	15	20					103	149
3 p.m.					119	6	77	93		

Constant diet: C. 324 gm., P. 156 gm., F. 159 gm., Na 204 mEq., K 115 mEq.

FIGURE 4. Comparison of the effects of hydrocortisone (100 mg.) and fluorohydrocortisone (2 mg.) administered to a normal subject in an 8-hour intravenous infusion in normal saline.

on a constant diet. The two compounds were administered in an eight-hour intravenous infusion in normal saline. It is apparent that the smaller dose of fluorohydrocortisone produced at least as much sodium retention as the larger dose of hydrocortisone. On the other hand, the eosinopenia produced by 100 mg. of hydrocortisone was considerably more marked than that produced by 2 mg. of fluorohydrocortisone.

Since a maximal electrolyte effect can be obtained with a relatively small dose of fluorohydrocortisone, it was of interest to ascertain whether a change in the glucocorticoid to mineralocorticoid activity ratio could be induced by the administration of relatively large doses of this compound. A 20-year-old female patient (FIGURE 5) was placed on a constant diet and the effect of 25 mg. of 9- $\alpha$ -fluorohydrocortisone by mouth was compared with that of a smaller dose, *i.e.*, five mg. daily. The effect on urinary sodium excretion was quite similar in the two studies, although maximal sodium retention was achieved more rapidly with the larger dose. The effect on urinary glucose excretion, however, was quantitatively quite different (FIGURE 5). The changed ratio of

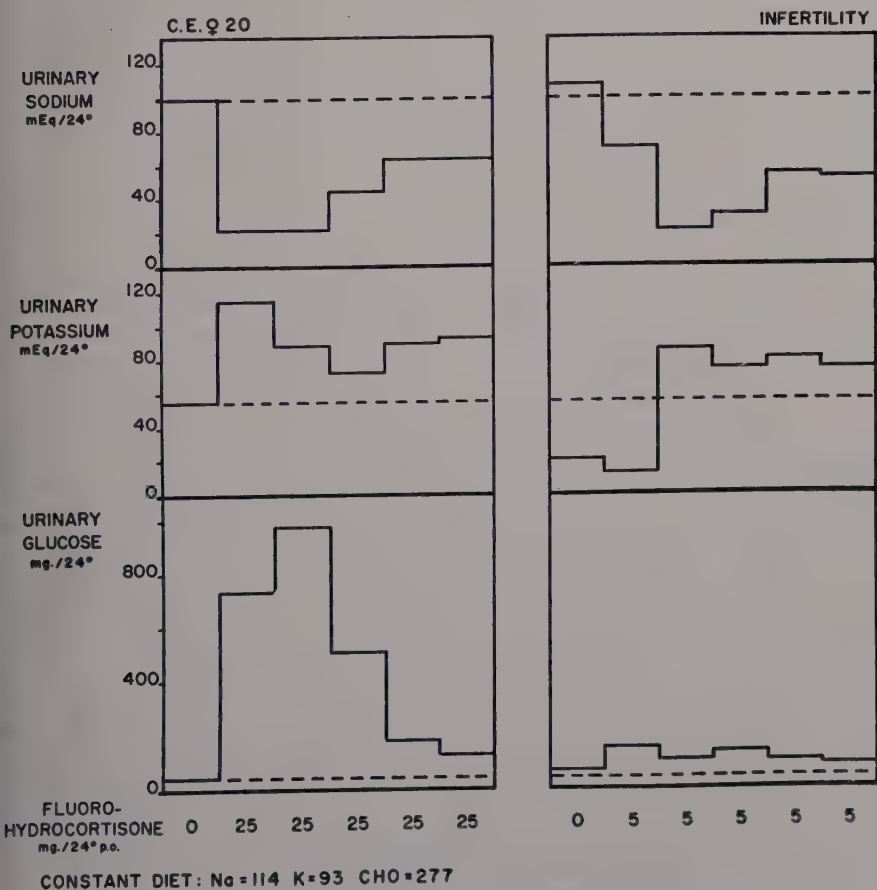


FIGURE 5. Effects of 9- $\alpha$ -fluorohydrocortisone.

mineralocorticoid to glucocorticoid activity at various dose levels may explain, in part, the observation that larger doses of fluorohydrocortisone, at times, give rise to no greater salt and water retention than small doses. Definite evidence of "escape" in the degree of sodium retention produced by the steroid was also seen at both dose levels (FIGURE 5). It is interesting to note that the effects of the steroid on urinary glucose excretion also showed definite evidence of an "escape."

The potentiating effect of the fluorine substitution in position 9-alpha does not appear to be specific for hydrocortisone but represents a more general effect. Studies in man confirm earlier observations in experimental animals.<sup>3</sup> Compounds which have been tested in this study include 9-alpha-fluoro-11-hydroxyprogesterone, 11,17-dihydroxyprogesterone, 11,21-dihydroxyprogesterone. Each of these compounds has been administered to patients with Addison's disease by the intravenous and oral route. Because of the small number of observations made to date, no definitive conclusions can as yet be reported. It would appear, however, that the potentiation of physiological activity obtained by the substitution of the fluorine atom in position 9-alpha was, in each instance, of the same order of magnitude. Thus, the intravenous infusion over an eight-hour period of *one mg.* of fluorocorticosterone was compared with that of *50 mg.* of corticosterone (FIGURE 6). It is quite apparent that the effect of the fluorinated corticosterone on the urinary sodium-to-potassium ratio was at least 50 times as marked as that of the nonfluorinated parent compound. A more prolonged action was also observed with the fluorinated compound. Further results have been reported by Goldfien.<sup>8</sup>

### Clinical Usefulness

The clinical indications for the use of 9-alpha-fluorohydrocortisone are suggested by its physiological properties. In the present studies, three areas of particular usefulness for 9-alpha-fluorohydrocortisone have emerged. The treatment of in-

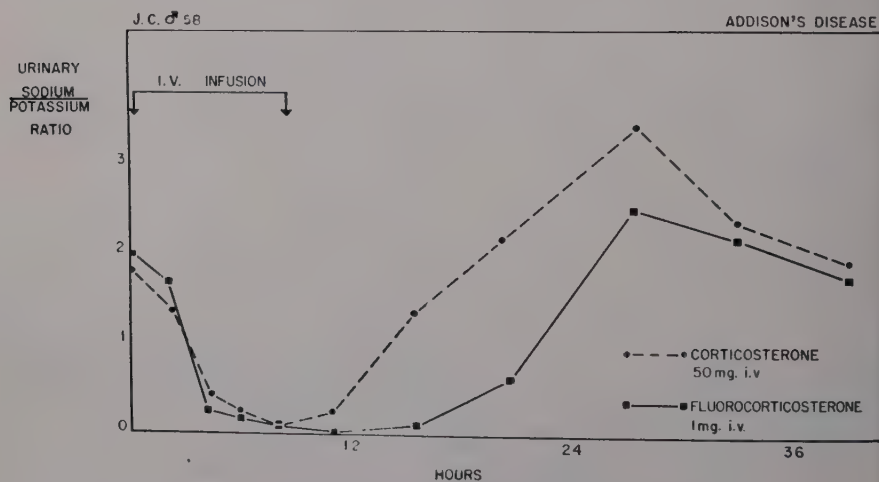


FIGURE 6. Effects of corticosterone and 9-alpha-fluorohydrocortisone.



flammatory diseases and the topical application of the compound are considered elsewhere in this monograph.

*Treatment of Addison's disease.* The prolonged duration of action and the increased mineralocorticoid activity, even when the compound is taken by mouth, suggest the use of 9 $\alpha$ -fluorohydrocortisone in the treatment of Addison's disease. A quantity of 0.25 to 0.5 mg. by mouth daily satisfies the requirements for salt-retaining hormone in patients with Addison's disease or in patients following bilateral adrenalectomy. It has thus become unnecessary to administer supplementary salt-retaining hormones by intramuscular injection. It is necessary, however, in almost all cases, to supplement this dose of fluorohydrocortisone with cortisone or hydrocortisone in order to achieve a feeling of maximum well-being for the patient. Between 6.25 and 25 mg. of cortisone acetate daily by mouth are usually adequate for this purpose.

*Inhibition of ACTH secretion by the pituitary.* The second area of usefulness of fluorohydrocortisone derives from its effectiveness as an inhibitor of ACTH secretion by the pituitary. It is of particular importance that the amounts required are relatively small and hence reduce to a minimum the quantity of urinary metabolites detectable by the Porter-Silber reaction. This fact greatly facilitates the interpretation of changes observed in the urinary excretion of endogenously secreted steroids. The effect of one mg. of fluorohydrocortisone daily, given as a single oral dose, on the urinary excretion of 17-hydroxycorticoids and 17-ketosteroids is illustrated in a male patient with normal adrenal function (FIGURE 7). It is apparent that the administration of fluorohydrocortisone resulted in a sharp drop in spontaneous 17-hydroxycorticoid excretion on the second day of therapy. A less impressive decrease in 17-ketosteroid excretion was observed from the third day onward. As might be anticipated, the response to a standard dose of intravenously administered ACTH decreased during fluorohydrocortisone inhibition. Similar results have been obtained in two other normal subjects.

This effectiveness of fluorohydrocortisone by mouth as an inhibitor of endogenous adrenal cortical secretion may be utilized both as a diagnostic and as a therapeutic tool. As a diagnostic tool, it may be used to differentiate between types of tissue responsible for hyperadrenocorticism. Cortisone and hydrocortisone have been previously used for this purpose.<sup>9, 10</sup> The high potency of fluorohydrocortisone, however, makes this substance more suitable than cortisone or hydrocortisone, since it minimizes the difficulties encountered in attempting to interpret changes in urinary 17-ketosteroids and 17-hydroxycorticoids when inhibition is induced by the administration of large quantities of cortisone and hydrocortisone, for these compounds contribute appreciably to both moieties. FIGURE 8 illustrates the inhibitory effect of fluorohydrocortisone in a patient with the adrenogenital syndrome. In this patient, the 17-ketosteroids, which had ranged from 23 to 26 mg. per day, decreased to 7 mg. per day after five days of 10 mg. of fluorohydrocortisone acetate. The hormone was given as a single daily dose by mouth. After 10 days of hormone therapy, the urinary 17-ketosteroid excretion attained a level of approximately 4 mg. per day. The prompt response of endogenously secreted hormone to fluorohydrocortisone medication strongly suggested that, in this instance, the

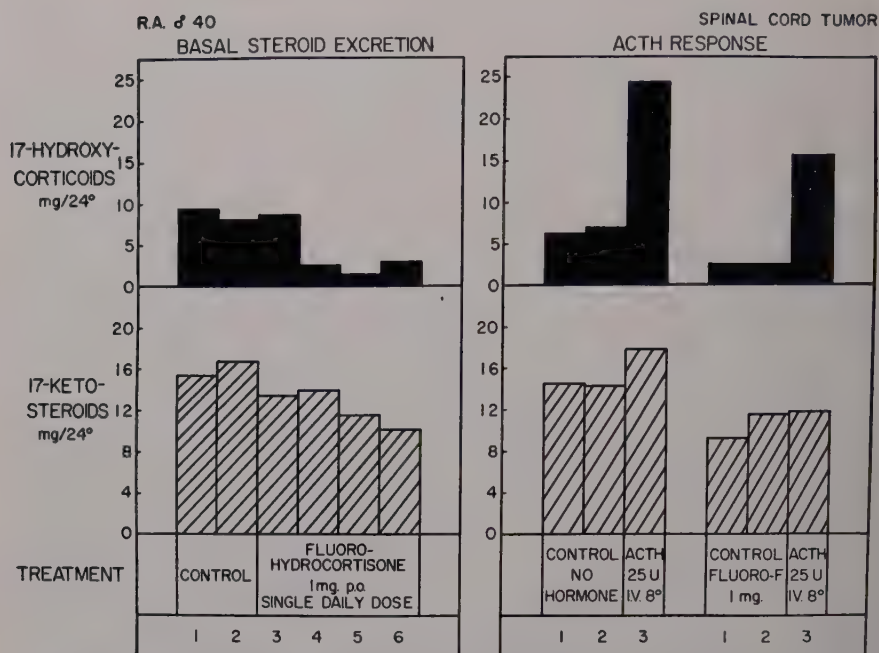


FIGURE 7. Effect of fluoro-hydrocortisone on basal steroid excretion and an ACTH response in a patient with normal adrenal function.

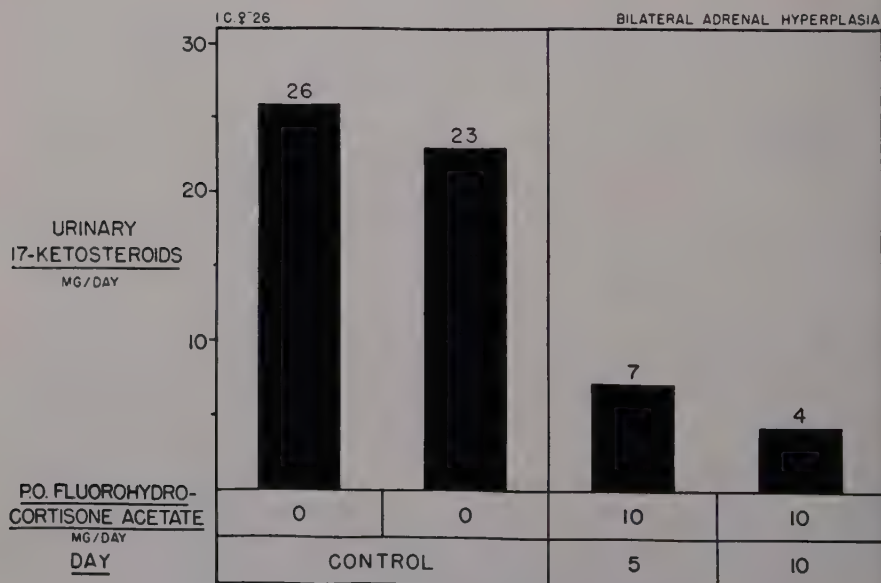


FIGURE 8. Adrenocortical inhibition with fluoro-hydrocortisone.

hormonal disturbance was due to bilateral adrenal cortical hyperplasia. This patient further illustrates the potential therapeutic usefulness of the compound, since this low level of hormone excretion has been maintained without untoward effects for a period of more than six months with a dose of only 2 mg. of steroid per day. In five other female patients with hirsutism and increased urinary 17-ketosteroid excretion, a dose of fluorohydrocortisone as small as one or two mg. per day given by mouth has been sufficient to maintain the 17-ketosteroid excretion at normal levels or below. While all patients exhibited some evidence of salt retention at the beginning of fluorohydrocortisone therapy, the supplementation of their diet with 6 grams of potassium chloride daily was usually sufficient to prevent excessive salt retention. Occasionally, sodium restriction in the diet was also necessary.

Fluorohydrocortisone therapy may also prove useful in patients in whom suppression of normal adrenal cortical secretion is indicated, *i.e.*, metastatic breast and prostatic cancer.

*Substitution for cortisone during tests of adrenocortical reserve.* A third general area of usefulness for the compound again derives from the fact that small quantities of fluorohydrocortisone are effective in maintaining patients with Addison's disease or may be substituted for much larger amounts of cortisone or hydrocortisone being administered over prolonged periods as anti-inflammatory therapy or for other reasons. Under these conditions, it is not necessary to discontinue steroid therapy in order to carry out definitive tests of adrenal cortical reserve function with ACTH. FIGURE 9 demonstrates the use

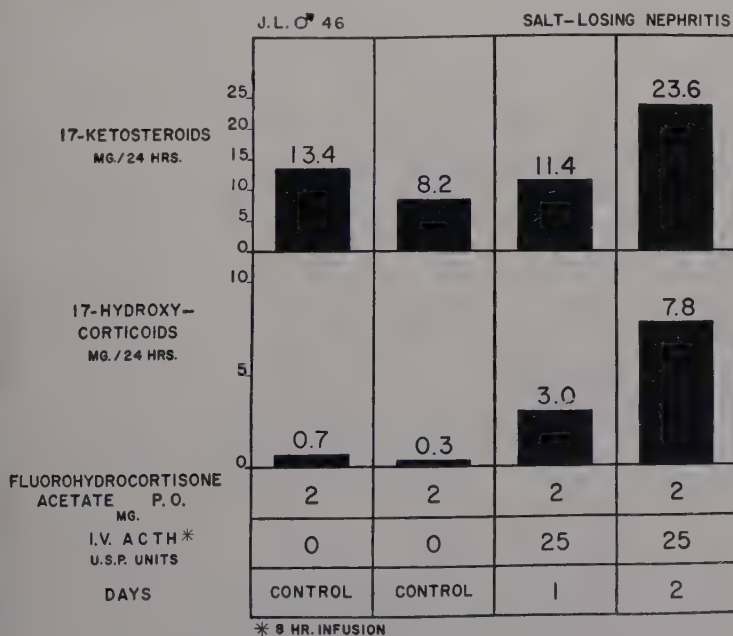


FIGURE 9. Adrenal response to intravenous ACTH in iatrogenic adrenal insufficiency.

of fluorohydrocortisone as substitution therapy for cortisone in a 46-year-old male who was admitted to the ward for evaluation of his adrenal cortical function. Two years previously, because of polyuria, acute salt depletion, and pigmentation, he had been started on 50 mg. of cortisone daily and on large doses of added salt, and this program had been continued for two years. Although the diagnosis of salt-losing nephritis appeared indubitable, the possibility of associated adrenal cortical insufficiency could not be readily dismissed. By substituting 2 mg. of fluorohydrocortisone for the daily dose of 50 mg. of cortisone, the urinary excretion of 17-hydroxycorticoids and 17-ketosteroids was rapidly brought to low levels. Activation of the adrenal cortex with ACTH stimulation was then demonstrated without risking the undesirable side effects which often accompany iatrogenic adrenal cortical atrophy.

### Summary

Studies in man have confirmed the fact that fluorohydrocortisone is a highly potent hydrocortisone derivative whose salt-retaining effects have been potentiated somewhat more than its glucocorticoid, or more specifically than its eosinopenic, glucosuric, catabolic, and anti-inflammatory action. The areas of clinical usefulness, which have been outlined, derive from its marked salt-retaining effects when administered by mouth, from its high degree of potency making it possible to obtain marked physiological effects in amounts giving rise to little or no interfering metabolites, and from its effectiveness as an inhibitor of ACTH secretion by the pituitary as measured by our indices.

### References

1. FRIED, J. & E. F. SABO. 1954. 9- $\alpha$ -fluoro derivatives of cortisone and hydrocortisone. *J. Am. Chem. Soc.* **76**: 1455.
2. BORMAN, A., F. M. SINGER & P. NUMEROF. 1954. Growth-survival and sodium retaining activity of 9- $\alpha$ -halo derivatives of hydrocortisone. *Proc. Soc. Exptl. Biol. Med.* **86**: 570-573.
3. FRIED, J. 1955. Biological effects of 9- $\alpha$  fluorohydrocortisone and related halogenated steroids in animals. *Ann. N. Y. Acad. Sci.* **61** (2): 573-581.
4. LIDDLE, G. W., M. M. PECHET & F. C. BARTTER. 1954. Enhancement of biological activities of corticosteroids by substitution of halogen atoms in 9 $\alpha$  position. *Science*. **120**: 496-497.
5. GOLDFIEN, A., J. C. LAIDLAW, N. ABU HAYDAR, A. E. RENOLD & G. W. THORN. 1955. Fluorohydrocortisone and chlorohydrocortisone, highly potent derivatives of compound F. *New Engl. J. Med.* **252**: 415-421.
6. RENOLD, A. E. & E. R. FROESCH. In preparation.
7. THORN, G. W., R. H. SHEPPARD, W. I. MORSE, W. J. REDDY, P. M. BEIGELMAN & A. E. RENOLD. 1955. Comparative action of aldosterone and 9- $\alpha$ -fluorohydrocortisone in man. *Ann. N. Y. Acad. Sci.* **61** (2): 609-619.
8. GOLDFIEN, A., W. I. MORSE, E. R. FROESCH, W. F. GANONG, A. E. RENOLD & G. W. THORN. 1955. Pharmacological studies in man of 11-, 17-, and 21-hydroxy derivatives of progesterone and their fluorinated analogs. *Ann. N. Y. Acad. Sci.* **61** (2): 433-441.
9. WILKINS, L., P. A. LEWIS, R. KLEIN, L. I. GARDNER, J. F. CRIGLER, JR., E. ROSENBERG & C. J. MIGEON. 1951. Treatment of congenital adrenal hyperplasia with cortisone. *J. Clin. Endocrinol.* **11**: 1-25.
10. JAILER, J. H., J. J. GOLD & E. Z. WALLACE. 1954. Evaluation of the "Cortisone Test" as a diagnostic aid in differentiating adrenal hyperplasia from adrenal neoplasia. *Am. J. Med.* **16**: 340-345.



## EXPERIENCES WITH 9-ALPHA-FLUOROHYDROCORTISONE ACETATE IN RHEUMATOID ARTHRITIS

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Although hydrocortisone and cortisone have earned important roles in the treatment of rheumatoid arthritis, the results from their long-term administration are less than satisfactory in a large proportion of patients. Widened experience with the hormones has led to refinements in methods for their practical application and has increased their utility—but it has also emphasized their inherent limitations. Chief among the latter, has been a proclivity to produce unwanted physiologic side effects in addition to the wanted antirheumatic action. The intrusion of undesirable reactions during the course of treatment has too often created a barrier to successful management, especially in patients with marked rheumatoid activity who require large daily doses for adequate control of the disease.

Thus, clinicians have continued to search for an agent which would provide a specialized anti-inflammatory action and would be devoid of annoying or deleterious effects. Hope that such a substance might be found in the steroid field has been fostered by the knowledge that it is possible to modify several properties of the hormones by substituting various chemical radicals or groupings at one or another carbon position in the steroid nucleus. Indeed, this fact has so excited the imagination of chemists that literally hundreds of synthetic analogs of hydrocortisone and cortisone have been prepared, and at a rate more rapid than they can be tested therapeutically or experimentally in animals.

Like others, we have screened a number of esterified preparations of hydrocortisone and have made clinical comparisons of their antirheumatic activity. In the spring of 1954, our attention was attracted to a halogen derivative—9-alpha-fluorohydrocortisone acetate—which, though not destined as a practical agent for systemic therapy in rheumatoid arthritis, was of interest because it exhibited far greater antirheumatic potency, milligram for milligram, than any steroid we had thus far tested.

Interest in the halogen derivatives of hydrocortisone was stimulated by results of animal studies which indicated that they possess unusually high glycogenic activity as measured by rat liver glycogen assays for 11-oxygenated corticoids. Fried and Sabo<sup>1, 2</sup> and Borman, Singer, and Numerof<sup>3, 4</sup> demonstrated that the 9-alpha-halo derivatives (chloro, fluoro, iodo, and bromo) manifest both glucocorticoid and mineralocorticoid properties, each in differing degrees. The glycogen deposition activity of the fluoro compound was found to be twice as great and its sodium-retaining effect to be only one-fifth that of the chloro derivative. Compared with cortisone acetate, fluorohydrocortisone acetate was much more potent in glycogenic activity (about 10 times), in producing thymus involution (four times), and in its sodium-retaining effect.

Data derived from clinical trials with 9-alpha-fluorohydrocortisone acetate

were meager. Thorn and his co-workers<sup>5</sup> had determined that the substance, given orally in extremely low dosage, was highly efficient in maintaining patients with Addison's disease. Dosages as small as 0.25 to 1 mg. a day were sufficient to hold patients in a near optimal state. Metabolic studies accomplished by them in Addisonian or bilaterally adrenalectomized patients demonstrated that the preparation caused marked retention of sodium and diuresis of potassium, and a striking and prolonged eosinopenic response. Comparative studies in a patient with Addison's disease disclosed that the compound's effect on electrolyte excretion was at least as powerful as that of aldosterone (electrocortin) and was more prolonged. In female patients with adrenogenital syndrome, 9-alpha-fluorohydrocortisone acetate, in doses as small as 5 mg. a day, caused marked reduction in urinary 17-ketosteroid excretion.

### *Clinical Trials in Rheumatoid Arthritis*

Nine-alpha-fluorohydrocortisone acetate\* was administered to 13 patients with active peripheral rheumatoid arthritis. The drug was given as initial investigative treatment to seven patients who had received no therapy other than conservative measures, for at least three months beforehand. Medication was later transferred to hydrocortisone (free alcohol) in three of these cases to allow dosage comparisons. Six patients, being maintained on established daily amounts of hydrocortisone (free alcohol) with satisfactory and stable clinical improvement, were transferred directly to the fluoro compound and differences in dosage requirements for approximately equivalent rheumatic control were estimated.

The drug was taken orally in divided doses, four times a day, following meals and at bedtime. Manipulations in dosage were made by increments and decrements of 0.5 or 1.0 mg. each. No complementary medication was prescribed, but the patients were kept on qualitatively sodium-poor diets. Three patients were observed under hospital conditions at the beginning, while the others were followed as ambulatory out-patients throughout the study. Decision to employ small total daily dosages was based on reported animal and clinical data which indicated that the derivative possessed extraordinary metabolic potency.

*Results in patients receiving 9-alpha fluorohydrocortisone acetate as initial suppressive therapy.* With initial suppressive dosages ranging from 4 to 8 mg. a day, subjective and objective improvement in the articular manifestations resulted in five of the seven patients (TABLE 1). Improvement was slight or questionable in one patient (L. D.), and another (A. J.) experienced an acute articular exacerbation involving multiple joints on the third day of treatment. In the five patients who demonstrated definite benefits, the onset and subsequent rate of improvement were rapid. The erythrocyte sedimentation rate was reduced promptly and strikingly in four of the seven patients. The speed and degree of over-all improvement in five of the patients compared favorably with that which might have been expected from hydrocortisone or from cortisone administered in much larger initial suppressive doses. These observations

\* Nine-alpha-fluorohydrocortisone acetate was supplied by The Squibb Institute for Medical Research, New Brunswick, N. J. Hydrocortisone (free alcohol) was supplied by Merck & Co., Rahway, N. J.

indicated that 9-alpha-fluorohydrocortisone acetate promotes potent anti-rheumatic effects when administered orally in very small total milligram doses per day.

*Comparisons of dosage requirements for 9-alpha-fluorohydrocortisone acetate and hydrocortisone (free alcohol).* Attempts were made in nine patients to estimate differences in milligram dosage requirements for the maintenance of similar degrees of rheumatic control (TABLE 2). One patient (M. H.), whose arthritis was controlled satisfactorily with 50 mg. of hydrocortisone (free alcohol) per day, was transferred to 9-alpha-fluorohydrocortisone acetate in amounts of 5 mg. a day and, four days later, she experienced a severe articular relapse. Dosage comparisons were not permitted in this case because marked fluid retention and moderate hypertension intervened, which prohibited trials with larger doses of the fluoro compound. From comparisons made in the remaining eight patients, the calculated dosage ratios of hydrocortisone (free alcohol) to 9-alpha-fluorohydrocortisone acetate ranged from 8.3:1 to 12.5:1. Thus it appeared from clinical observations alone that the milligram antirheumatic potency of 9-alpha-fluorohydrocortisone acetate was, roughly, ten times greater than that of hydrocortisone (free alcohol).

*Adverse reactions.* During the short periods of observation embraced by the investigation, and with the dosage range explored, two adverse reactions to 9-alpha-fluorohydrocortisone acetate were prominent: fluid retention and blood pressure elevation.

Retention of fluid, as indicated by edema and/or sudden gains in weight, was noted in 12 of the 13 patients. It was graded as marked in 4, moderate in 5, and slight in 3. In most instances, it began after two to six days of administration and, when pronounced, was accompanied by weight increases as great as 1 to 2 lb. per day. In order that this effect could be observed uninfluenced by countermeasures, diuretics were not employed. When transfer of medication to hydrocortisone (free alcohol) was made in larger but equally efficient amounts, signs of fluid retention disappeared quite promptly, ordinarily within two to five days. These observations suggested that the substitution of a fluorine atom at the ninth carbon position of hydrocortisone not only enhanced the anti-inflammatory activity of the steroid but increased its salt-and water-retaining property to an even greater degree.

Blood pressure elevations were recorded in 6 of the 13 patients, these being graded as slight in 3 and moderate in 3. In each instance where transfer of medication to hydrocortisone (free alcohol) was made, the blood pressure returned to its former level within 4 to 14 days.

The periods of 9-alpha-fluorohydrocortisone acetate administration were not sufficiently long to permit assessment of other possible endocrine side effects from the drug. One patient (S. S.) who received the preparation in maintenance dosages of 4 mg. a day for a total of 77 days exhibited faint facial mooning after four weeks of administration.

Two unusual reactions were observed. One patient (R. B.) suddenly experienced dryness of the mouth, a "toxic" feeling, and low-grade fever after the fluoro compound had been taken for 14 days. The symptoms disappeared

TABLE 1  
CLINICAL RESPONSE OF PATIENTS WITH RHEUMATOID ARTHRITIS TO INITIAL SUPPRESSIVE DOSES OF 9-ALPHA-FLUOROHYDROCORTISONE ACETATE ADMINISTERED ORALLY

Patient	Age (years)	Sex	Disease duration (years)	Overall disease severity (activity)	Initial dosage (mg. per day)	Onset of improvement (hours after first divided dose)	Degree and rapidity of initial clinical improvement	Erythrocyte sedimentation rate (Westergren method) (mm. in 1 hour)		Duration of administration (days)	Fluid retention		Other effects; miscellaneous comment
								Before administration	Rapidity of change with administration		Objective edema	Sudden weight gain	
1. CM	55	F	4	Severe	4 × 2d; 6 × 2d; 8 × 4d; then 7 mg. a day as maintenance dose	17 (after total of 3 mg.)	Marked in 6 days	96	29 after 8 days	81	Moderate to slight (variable)	None	Slight elevation of blood pressure
2. SS	48	F	7	Moderately severe	6 × 7d; 5 × 3d; then 4 mg. a day as maintenance dose	6 (after total of 2 mg.)	Marked in 13 days	58	25 after 10 days	77	Slight	10 lbs. during first 10 days on doses of 6 and 5 mg./day; no further gain on 4 mg./day	Moderate elevation of blood pressure; moderate insomnia on 6 mg./day; slight on 4 mg./day; slight facial mooning after 4 weeks administration; after 8 weeks irregular nausea noted
3. LC	59	F	8	Moderate	6 × 5d; 5 × 3d; 4 × 5d; 3 × 8d; then transfer to hydrocortisone	7 (after total of 3 mg.)	Very marked in 7 days	49	16 after 10 days	21	Marked on doses of 6 and 5 mg./day; moderate on doses of 3 mg./d.	13 lbs. during first 13 days on doses of 6, 5, and 4 mg./day	Moderate elevation of blood pressure. After transfer to hydrocortisone (free alcohol) evidences of fluid retention disappeared within 5 days and blood pressure returned to pre-treatment level in 14 days
LB	55	F	5	Moderately severe	4 × 21d; then transfer to hydrocortisone	30	Marked in 14 days	44	16 after 10 days	21	Moderate	4½ lbs. during first 5 days; then no further gain	Slight elevation of blood pressure. After transfer to hydrocortisone (free alcohol) evidences of fluid retention disappeared and blood pressure returned to normal in 5 days
EA	65	M	1	Moderate	4 × 14d; then transfer to hydrocortisone	24	Marked in 14 days	47	34 after 14 days	14	Moderate	10 lbs. in 14 days	Evidences of fluid retention disappeared 4 days following transfer to hydrocortisone (free alcohol)
LD	58	F	½	Moderately severe	4 × 4d; 6 × 4d; 7 × 5d; then transfer to hydrocortisone	?	Slight in 13 days	65	48 after 13 days	13	None	None	Improvement only slight, even questionable; definite improvement later, following transfer to hydro-



7. AJ	53	M	4	Moderate	4 X 7d; then discontin- ance	Worse after 72 hours	Worse	33	23 after 7 days	7	Moderate	5 lbs. in 7 days	Acute articular flare-up after 72 hours of adminis- tration with subsidence following withdrawal. History of similar exacer- bation after 1 week of cor- tisonone therapy 2 years previously. Refused trial on hydrocortisonone
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TABLE 2  
COMPARISONS OF MAINTENANCE DOSAGE REQUIREMENTS FOR 9-ALPHA-FLUOROHYDROCORTISONE ACETATE AND HYDROCORTISONE (FREE ALCOHOL) ADMINISTERED ORALLY IN PATIENTS WITH RHEUMATOID ARTHRITIS

Patient	Disease severity (activity)	Degree overall improvement maintained with both compounds	Daily milligram dosages required for approximately equivalent degrees of rheumatic control		Calculated dosage ratios: Hydrocortisone (free alcohol) to 9-alpha-fluorohydrocortisone acetate	Fluid retention while on respective maintenance doses		Blood pressure elevations on respective maintenance doses		Miscellaneous comment
			Hydrocortisone (free alcohol)	9-alpha-fluorohydrocortisone acetate		Hydrocortisone (free alcohol)	9-alpha-fluorohydrocortisone acetate	Hydrocortisone (free alcohol)	9-alpha-fluorohydrocortisone acetate	
1. LC	Moderate	Very marked	25	3	8.3 to 1	None	Moderate	Slight	Moderate	Nausea with 9-alpha-fluorohydrocortisone acetate; none with hydrocortisone (free alcohol) After 2 weeks of 9-alpha-fluorohydrocortisone acetate administration developed sudden dryness of mouth, "toxic feeling," and low-grade fever; these disappeared 2 days after transfer to hydrocortisone (free alcohol) After 13 days of 9-alpha-fluorohydrocortisone acetate administration developed abdominal bloating, moderate facial edema, gr. 2 albuminuria without azotemia; symptoms disappeared 4 days after transfer to hydrocortisone (free alcohol)
2. LB	Moderately severe	Marked	40	4	10.0 to 1	None	Moderate	None	None	
3. EA	Moderate	Marked	40	4.5	8.9 to 1	None	Moderate	None	None	
4. CH	Moderately severe	Very marked	30	3	10.0 to 1	None	Slight	None	Slight	
5. RB	Moderately severe	Marked	50	5	10.0 to 1	None	Moderate	None	Moderate	
6. OD	Moderately severe	Marked	35	4	8.8 to 1	None	Marked	None	None	Pronounced articular relapse 4 days following transfer to 9-alpha-fluorohydrocortisone acetate. Fluid retention and blood pressure elevation disallowed trial of larger doses
7. CR	Moderately severe	Marked	50	4	12.5 to 1	None	Marked	None	None	
8. GH	Moderate	Marked on hydrocortisone	35	3.5	10.0 to 1	None	Slight	None	Slight	
9. MH	Moderately severe	Relapse on fluorohydrocortisone	50	? (greater than 5)	—	Slight	Marked	Slight	Moderate	

two days after resumption of hydrocortisone (free alcohol) therapy. Another patient (O. D.) developed abdominal bloating, moderate facial edema, and grade 2 albuminuria with cylindruria after 13 days of medication. These reactions were corrected within five days following transfer to hydrocortisone (free alcohol). Two patients complained of slight, intermittent nausea.

### *Comment*

The cases studied were too few and the periods of observation were too short to allow more than provisional deductions. It was obvious, however, that the powerful salt- and water-retaining properties of 9-alpha-fluorohydrocortisone and, perhaps, other metabolic effects which might ensue with prolonged administration would prohibit its application as systemic therapy for rheumatoid arthritis. Dosages in the order of 3 to 8 mg. a day were required to provide adequate suppression of rheumatic manifestations when the disease was of moderate or greater activity. Even with such small amounts, most of the patients developed evidences of fluid retention and, in some, this complication was pronounced and intolerable. The fact that the fluid retention disappeared promptly when treatment was changed to hydrocortisone (free alcohol) in much larger, but equally efficient antirheumatic doses, indicated that the substitution of a fluorine atom at the ninth carbon position of hydrocortisone caused proportionately even greater potentiation of its salt-retaining effect than of its antiphlogistic action.

These observations are of interest chiefly because they demonstrate that the anti-inflammatory potency of hydrocortisone may be enhanced, and indeed multiplied, from an alteration in its formula. When this is considered, together with the knowledge that other properties of the hormone also may be modified by chemical substitutions at certain carbon positions of the steroid nucleus, hope is raised that synthetic analogs may be prepared which will have a higher therapeutic index—*i.e.*, compounds with more selective anti-inflammatory activity and with fewer or less significant undesirable effects.

### *Summary*

A halogenated derivative of hydrocortisone—9-alpha-fluorohydrocortisone acetate—was administered as investigative therapy to 13 patients with active rheumatoid arthritis. Seven patients received the preparation as initial medication. Three of these were transferred later to hydrocortisone (free alcohol) for comparisons of dosage requirements. Six patients, being maintained on established daily amounts of hydrocortisone (free alcohol), were transferred directly to the fluoro compound and comparisons of the milligram doses needed for similar degrees of rheumatic control were made.

The following conclusions may be drawn from the clinical observations:

(1) Weight for weight, the antirheumatic potency of 9-alpha-fluorohydrocortisone acetate was found to be much greater than that of the parent compound, hydrocortisone. This was indicated by the following: (a) initial suppressive doses of the fluoro derivative ranging from 3 to 8 mg. a day were sufficient, in five of seven patients, to promote rapid improvement in the rheumatic manifestations; (b) comparisons of maintenance dosage requirements

for approximately equivalent degrees of clinical control revealed that, in eight of nine patients, the antirheumatic power of 9- $\alpha$ -fluorohydrocortisone acetate, milligram for milligram, was roughly 10 times that of hydrocortisone (free alcohol).

(2) With the small total daily amounts of the fluoro compound employed, signs of fluid retention developed in 12 of the 13 patients, and it was pronounced in some. This suggested that the substitution of a fluorine atom at the ninth carbon position increased the electrolyte activity of hydrocortisone to an extent proportionately greater than it enhanced its antiphlogistic property. The excessive salt- and water-retaining effect of the fluoro derivative would seem to preclude its practical application as systemic therapy for rheumatoid arthritis.

The findings are of importance chiefly because they demonstrate that it is possible to enhance greatly the antiphlogistic action of hydrocortisone, as well as to modify other of its properties, by altering its chemical formula. This potentiality raises hope that synthetic steroids may be produced in the future that may be applied more successfully in the treatment of rheumatoid arthritis and other responsive diseases.

### *References*

1. FRIED, J. & E. F. SABO. 1953. Synthesis of 17  $\alpha$ -hydroxycorticosterone and its 9- $\alpha$ -halo derivatives from 11-epi-17  $\alpha$ -hydroxycorticosterone. *J. Am. Chem. Soc.* **75**: 2273.
2. FRIED, J. & E. F. SABO. 1954. 9- $\alpha$  fluoro derivatives of cortisone and hydrocortisone. *J. Am. Chem. Soc.* **76**: 1455.
3. BORMAN, A. & F. M. SINGER. 1954. Adrenocortical activity of 9  $\alpha$ -halo derivatives of cortisone and hydrocortisone. *Federation Proc.* **13**: 185.
4. BORMAN, A., F. M. SINGER & P. NUMEROF. 1954. Growth survival and sodium retaining activity of 9  $\alpha$ -halo derivatives of hydrocortisone. *Proc. Soc. Exptl. Biol. Med.* **86**: 570.
5. GOLDFIEN, A., J. C. LAIDLAW & G. W. THORN. Chlorohydrocortisone and fluorohydrocortisone. Highly potent derivatives of compound F. *New Engl. J. Med.* In press.



# COMPARATIVE EFFECTIVENESS OF FLUOROHYDROCORTISONE AND HYDROCORTISONE IN THE TOPICAL TREATMENT OF SKIN DISEASES\*

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The living human skin probably excels all other organs as an instrument for assaying certain effects of agents intended for use upon man. For the skin offers a vast expanse and volume of highly differentiated and actively functioning living tissue of easily accessible and, to a great degree, "expendable," human tissue, upon which the action of all manner of physical agents and chemical compounds can be tested with relative safety, economy, and dispatch. Moreover, such direct dermatologic "bioassays" upon human structures (which one might term "bioanthropologic assays") offer decisive advantages, of course, over bioassays upon lower laboratory animals (which one might term "biozoologic assays"). For there is surely no thoughtful medical investigator who does not today recognize the wide, often diametric differences in species response—and their sometimes dire consequences when one attempts to apply to man the therapeutic, toxicologic, or other biologic results obtained in experiments upon laboratory animals.

The study of ACTH and of the corticosteroids has once more demonstrated the special advantages of dermatologic assays utilizing the human skin and its lesions. Prominent among these advantages is the fact that selected responsive skin lesions will often be seen to improve or even disappear, within a few hours to a day or so, after an effective dose of the hormone is reached; and to get worse or recur within a few hours to a day or so after the dose of hormone falls below the effective level. This suppression and exacerbation of visible skin lesions can often be produced and utilized over and over again in the same patient. Moreover, numerous observations of their responses have shown that human skin lesions are often precise and sensitive indicators, so that a few milligrams more or less in the daily dose of hormone not uncommonly determines the presence or absence of therapeutic effects.

To these advantages in studying systemically administered hormones, there are still additional ones that make their appearance when skin lesions are used to assay the hormones which are applied *externally* as agents of *topical therapy*. For example, employing a dermatologic procedure which we have called the "method of simultaneous symmetrical paired comparisons,"<sup>1</sup> one can directly and readily compare different topically applied agents and can rapidly obtain statistically valid data as to their relative efficacy. In this procedure, patients are selected who have symmetrically situated skin lesions of closely similar duration, degree, and extent. The lesions on one side are then treated with

\* I wish to thank Doctor Harvey Blank of The Squibb Institute for Medical Research, New Brunswick, N. J., for first calling 9- $\alpha$ -fluorohydrocortisone to our attention and for supplying us with the preparations used in these studies. We are grateful also to Doctor Elmer Alpert of Merck and Co. Inc., Rahway, N. J., and to Doctor H. F. Hailman of the Upjohn Company, Kalamazoo, Mich., for later supplies of the hormone in various vehicles.

TABLE 1\*

COMPARISON OF DOSES OF ACTH AND CORTISONE ESTIMATED TO BE THERAPEUTICALLY OF APPROXIMATELY EQUAL EFFICACY

	ACTH by slow intravenous drip	ACTH intramuscularly in absorption-delaying vehicle (e.g. gel)	ACTH intramuscularly in aqueous solution	Cortisone by mouth or by intramuscular injection†
	milligram	milligram	milligram	milligram
Approximate therapeutic equivalents in milligrams as established in various dermatoses.....	1‡	2	5-10	10-40
Average initial suppressive dose based on the above approximate therapeutic equivalents.....	30	60	150	300
Average maintenance dose based on the above approximate therapeutic equivalents.....	10	20	50	100

\* From Sulzberger, M. B. *Modern Trends in Dermatology*, Second Series, p. 336.

† Cortisone esters are now obtainable for intravenous administration. At the time of writing, the information as to relative biologic effectiveness was not available to the author.

‡ One milligram equals one international unit.

one agent and those on the other side with another agent. Of course this method of paired comparisons permits many variations designed to meet the requirements of the particular assay. Thus a given active ingredient in a particular vehicle can be compared with the particular vehicle alone, or an unknown "new" agent can be compared with a standard agent of known effectiveness, or different concentrations of the same agent can be compared with each other, or a given agent and concentration in one vehicle can be compared with the same agent and concentration in another vehicle, *etc.*

Many dermatologists, including those of our group at New York University-Bellevue Medical Center and the Skin and Cancer Unit of University Hospital, during recent years, have put to good use the advantages offered by skin lesions for the study of ACTH in various injectable forms and preparations, and also for assaying various systemically administered corticosteroids. I shall not go into further detail here, but merely submit, in tabular form, our estimates of the approximate relative efficacy of some of the systemically administered preparations, based mainly upon the studies of Bloom, Sobel, and Pelzig in cases of pemphigus and of erythrodermas in our wards at Bellevue Hospital.<sup>2-6</sup>

It was even more natural, of course, that dermatologists should assay upon human skin lesions the effectiveness of topically applied corticosteroids as rapidly as the various derivatives became available for clinical study. The results of these assays form the subject of many published reports.<sup>7-13</sup> Our own results, obtained not exclusively, but in great measure by the method of simultaneous symmetrical paired comparisons, have been corroborated by a majority of other investigators. These results may be briefly summarized as follows:

- (1) Cortisone acetate, in general, is quite ineffective on topical application.
- (2) Topical applications of hydrocortisone acetate, hydrocortisone free al-

cohol, and chlorohydrocortisone are generally effective in many cases of selected common dermatoses, *e.g.*, in atopic dermatitis (of all ages, including infantile eczema), in various forms of eczematous and eczematoid dermatoses, in otitis externa, and in anogenital pruritus.

(3) The vehicles in which the hydrocortisone is incorporated are sometimes of decisive importance, lotions and emulsions being advantageous in some cases (particularly those with extensive eruptions). Greasy ointments are preferable in others (especially in dry and scaly eruptions), and water-miscible creams are superior in some cases of moist eruptions or of dermatoses in moist areas.

(4) The effective concentrations of hydrocortisone acetate range from 0.25 per cent to 5 per cent and more. In most cases, however, optimum responses can be achieved by concentrations of between 1 per cent and 2.5 per cent.

It was against this background of experience and with similar methods of comparison that we began our studies of topical applications of 9 $\alpha$ -fluorohydro-

TABLE 2\*

TOPICAL FLUOROHYDROCORTISONE ACETATE COMPARED WITH HYDROCORTISONE FREE ALCOHOL (H.F.A.)

No. of cases	Diagnosis	Fluorhydrocortisone acetate					No simultaneous control (only fluorohydrocortisone acetate used)
		Much better than H.F.A.	Slightly better than H.F.A.	Equally effective	Equally in-effective	Not as good as H.F.A.	
28	Atopic dermatitis	5	10	11		2	
7	Allergic eczematous contact dermatitis	2	2	1		1	1—Improvement
3	Nummular eczema	1	1		1		
2	Lichen simplex chronicus			2			
2	Pruritus vulvae						2—No improvement
2	Otitis externa					1	1—Improvement
2	Intertrigo		1				1—No improvement
3	Lichen planus		1		1		1—Improvement?
3	Psoriasiform dermatitis		2	1			
1	Perivulvar psoriasis		1				
2	Psoriasis						2—Improvement?
3	Erythroderma		2	1			
1	Seborrheic dermatitis						1—Improvement
1	Dermatitis medicamentosa					1	
1	Alopecia areata						1—No improvement
1	Pemphigus vulgaris						1—No improvement
62	Totals	8	20	16	2	5	11

\* From Witten, Sulzberger, Zimmerman & Shapiro. 1955. J. Investigative Dermatol. In press.

cortisone shortly after this compound was prepared by Fried and Sabo in 1953<sup>14, 15</sup> and became available to us early in May 1954.

Based upon the earlier findings of other investigators who had studied its action and toxicity in systemic administration in laboratory animals and in man,<sup>16</sup> it seemed that this particular halogenated derivative might prove to be more potent than the earlier hydrocortisone preparations. TABLES 2 and 3 present the results of our direct comparisons on the cases studied and tabulated to date.<sup>17</sup> They permit the inference that, in the concentrations studied, the fluorohydrocortisone was topically more active than the nonfluorinated compound in a substantial proportion of the cases treated (28 of 41—TABLE 2). In many instances, it may be used effectively in one tenth of the concentra-

TABLE 3

COMPARISON OF 9 $\alpha$ -FLUOROHYDROCORTISONE ACETATE OINTMENT, 0.1 AND 0.25 PER CENT WITH HYDROCORTISONE (FREE ALCOHOL) OINTMENT, 2.5 PER CENT (Vehicle—Lanolin, Liquid Petrolatum, and Petrolatum)

Patient	Diagnosis	Duration of disease	Estimated improvement		
			Fluoro. F. Ac.		F (OH) 2.5%
			0.1%	0.25%	
J. R.	Atopic dermatitis	7 years	0	+++	0
B. B.	Atopic dermatitis	14 years	0		++
H. F.	Atopic dermatitis	2.5 years		0	++
R. Z.	Atopic dermatitis	26 years	0		0
R. W.	Atopic dermatitis	8 years		+++	++
T. B.	Atopic dermatitis	5 years	+++++		++++
S. S.	Atopic dermatitis	10 years	++		++
R. R.	Atopic dermatitis	6 years		+++	++
M. T.	Atopic dermatitis	44 years		+++	++
J. H.	Atopic dermatitis	24 years	0		+
G. E.	Atopic dermatitis	24 years	+		0
S. A.	Atopic dermatitis	29 years	0		+
E. A.	Allergic eczematous contact dermatitis	11 days	+++++		++++
A. N.	Allergic eczematous contact dermatitis	3 years	+		+
P. A.	Nummular eczema	4 years		+++++	++
M. S.	Seborrheic dermatitis	20 years	+		+
O. S.	Seborrheic dermatitis	5 weeks		+++	+++
E. A.	Intertrigo	6 mos.		+++++	N. C.
R. A.	Dermatophytid	2 weeks	+		+
H. K.	Folliculitis (seborrheic dermatitis)	14 years		0	0

## Summary

Total: 20 patients

Ages: 3 to 71 years

Duration of comparative hydrocortisone treatment: 1 day to 4 weeks (average 2.4 weeks)

Fluorohydrocortisone better than hydrocortisone (free alcohol):..... 8

Hydrocortisone (free alcohol) better than fluorohydrocortisone:..... 4

Fluorohydrocortisone equal to hydrocortisone (free alcohol):..... 7

No change:..... 1



tion found necessary when applying the hydrocortisone preparations we had studied previously (TABLE 3). This ratio of effectiveness corresponds very closely to the biologic activities reported in the assays upon laboratory animals and in studies of systemic administration in patients with Addison's disease or with adreno-genital syndrome.<sup>16</sup>

It is not known why fluorohydrocortisone is in many cases more effective in external dermatologic therapy than chlorhydrocortisone or hydrocortisone itself. It is possible, however, that differences in the size of the molecule, its solubilities, affinities, *etc.*, may bring about differences in rate of penetration and/or in concentration and persistence in the cutaneous structures. Such factors may lead to differences in therapeutic efficacy.

It is noteworthy that, as in the case of all the earlier hydrocortisone compounds we have studied,<sup>7-18</sup> there was no single observation in our patients proving or even suggesting systemic effects from the topical applications of fluorohydrocortisone. Furthermore, as in the previously studied compounds, there was no case of allergic eczematous or other sensitization to the fluorohydrocortisone. From our results to date,<sup>19</sup> the fluoro compound would appear to be both very effective and quite safe when used in small quantities in generally healthy persons.\*

### *Summary and Inferences*

(1) Mention is made of some of the many advantages offered by the living human skin and its lesions for assaying the effects of agents intended for human use.

(2) This form of dermatologic "bioanthropologic assay" has already proved its value for evaluating the relative effectiveness of systemically administered preparations of ACTH and of various corticosteroids.

(3) The human skin and its lesions offer additional advantages for evaluating the effects of topically applied medicaments, in particular, through the use of paired comparisons of different agents simultaneously applied to symmetrically situated sites and skin lesions.

(4) These methods of dermatologic "bioanthropologic assay" have proved that cortisone has little or no therapeutic action when topically applied in selected dermatoses, while hydrocortisone (acetate or free alcohol) and chlorohydrocortisone (acetate and free alcohol) are therapeutically effective in many cases of the same selected skin diseases.

(5) In the 82 cases of dermatoses included in this report, further studies employing the same methods have indicated that, in one tenth the concentration, topical *fluorohydrocortisone* acetate is, in many, though not all instances, substantially as effective as a topical agent than hydrocortisone free alcohol. Since hydrocortisone free alcohol is usually as effective as, or more effective than, other previously studied derivatives, it may now be inferred that fluorohydrocortisone is, in general, the most effective of the topical hydrocortisone compounds our group has studied to date.

(6) No systemic absorption, no systemic effects, and no instances of allergic sensitization have been observed by us following the topical application of any

\* Since the preparation of this report, Clarence Livingood and others have shown that systemic effects, in particular salt and water retention, can follow the topical use of fluorohydrocortisone, especially in lotion form. This finding suggests the need for great caution in its use, notably for extensive dermatoses in patients with possible renal, cardiovascular, or pulmonary weaknesses.

of the hydrocortisone derivatives. The nonhalogenated compounds in particular appear to be both very safe and effective and very practical topical remedies. In our hands they have thus far proved themselves to be among the safest of external remedies, eminently suitable for either short or long-term use, and for repeated applications to either small or extensive areas of damaged skin.

### References

1. SULZBERGER, M. B., R. L. BAER, A. KANOF & C. LOWENBERG. 1946. Methods for the rapid evaluation of the beneficial and harmful effects of agents applied to the human skin. *J. Investigative Dermatol.* **7**: 5.
2. BLOOM, D., N. SOBEL & A. PELZIG. 1953. Corticotropin and cortisone. Dosage and ratio of intramuscular and intravenous corticotropin (ACTH) and oral cortisone in treatment of certain dermatoses. *Arch. Dermatol. and Syphilol.* **67**: 61-65.
3. SULZBERGER, M. B. 1952. Cortisone and corticotropin (ACTH): their effects on the skin and its diseases. : 145. III. *Proc. 10th Intern. Congr. Dermatol.* London, England.
4. SULZBERGER, M. B. 1954. Cortisone and ACTH in dermatology. : 296. *Modern trends in Dermatology*, Second Series. Butterworth. London, England.
5. SULZBERGER, M. B., V. H. WITTEN & S. N. YAFFE. 1951. Cortisone acetate administered orally in dermatologic therapy. *Arch. Dermatol. and Syphilol.* **64**: 573-579.
6. SULZBERGER, M. B. & R. L. BAER. 1952. Present status of ACTH, cortisone and compound F in dermatologic management. *Year Book of Dermatol. and Syphilol.* Year Book Publishers. Chicago, Ill.
7. SULZBERGER, M. B. & V. H. WITTEN. 1952. The effect of topically applied compound F in selected dermatoses. *J. Investigative Dermatol.* **19**: 2.
8. SULZBERGER, M. B., V. H. WITTEN & C. C. SMITH. 1953. Hydrocortisone (compound F) acetate ointment in dermatological therapy. *J. Am. Med. Assoc.* **151**: 468-472.
9. SULZBERGER, M. B. & V. H. WITTEN. 1954. Hydrocortisone ointment in dermatological therapy. *Med. Clinics N. Amer.* **38**(2): 321.
10. WITTEN, V. H., A. B. AMLER, M. B. SULZBERGER & A. G. DESANCTIS. 1954. Hydrocortisone ointment in the treatment of infantile eczema. *Am. J. Diseases Children.* **87**: 298-304.
11. SIDI, E., V. BOURGEOIS-GAVARDIN & G. PLAS. 1953. Topical application of hydrocortisone acetate in treatment of eczema and pruritus. *Presse méd.* **61**: 992.
12. ROBINSON, H. M. & R. C. V. ROBINSON. 1954. Treatment of dermatoses with local application of hydrocortisone acetate. *J. Am. Med. Assoc.* **155**: 1213.
13. MALKINSON, F. D. & G. C. WELLS. 1954. Clinical experience with hydrocortisone ointment. *Brit. J. Dermatol.* **66**: 300.
14. FRIED, J. & E. F. SABO. 1953. Synthesis of 17<sub>a</sub>-hydroxycorticosterone and its 9<sub>a</sub>-halo derivatives from 11-epi-17<sub>a</sub>-hydroxycorticosterone. *J. Am. Chem. Soc.* **75**: 2273.
15. FRIED, J. & E. F. SABO. 1954. Fluoro derivatives of cortisone and hydrocortisone. *J. Am. Chem. Soc.* **76**: 1455.
16. GOLDFIEN, A., P. M. BEIGELMAN, J. C. LAIDLAW & G. THORN. 1954. (Abstract). Chlorhydrocortisone—a highly potent derivative of compound F. *J. Clin. Endocrinol.* **14**: 782.
17. WITTEN, V. H., M. B. SULZBERGER, E. H. ZIMMERMAN & A. J. SHAPIRO. 1955. A therapeutic assay of topically applied 9  $\alpha$ -fluorohydrocortisone acetate in selected dermatoses. *J. Investigative Dermatol.* In press.
18. SMITH, C. C. 1953. The eosinophilic response after inunction of hydrocortisone ointment; experiment demonstrating lack of significant absorption and of systemic effects. *Arch. Dermatol. and Syphilol.* **68**: 50.
19. WITTEN, V. H., A. J. SHAPIRO & R. H. SILBER. 1955. Studies on the inunction of hydrocortisone ointment (an attempt to demonstrate absorption by means of a new chemical test for hydrocortisone in body fluids). Submitted for publication to *Proc. Soc. Exptl. Biol. Med.*

## B. ALDOSTERONE

### THE POSSIBLE ROLE OF ALDOSTERONE IN EDEMA\*

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Suppression of sodium excretion in the urine accompanies the accumulation of edema in cardiac failure, the nephrotic syndrome, hepatic cirrhosis, and toxemia of pregnancy. This pattern of impaired sodium excretion occurs in the presence of low, normal, or even increased glomerular filtration rates. In addition, when changes in glomerular filtration and renal blood flow have an effect on sodium excretion, the acute initial increase or decrease in sodium output is often less striking if renal circulatory changes are prolonged. Although increased capillary or venous hydrostatic pressure and diminished plasma oncotic pressure may play an important role, they are not essential for edema formation.

The observation of Luetscher, Hall, and Kremer<sup>1</sup> that, in the nephrotic syndrome, after administration of concentrated human serum albumin, very large changes in glomerular filtration were accompanied only by small and sluggish changes in sodium excretion, indicated the predominance of increased sodium reabsorption by the renal tubule. Since desoxycorticosterone was a prototype of steroids with a maximal effect on renal tubular reabsorption of sodium, these observations stimulated the search for such a factor in the formation of edema. Lipid extracts of the urine of certain edematous patients with cardiac and renal disease were reported in 1950 by Deming and Luetscher<sup>2</sup> to have abnormally high sodium-retaining potency. These urine extracts had an effect comparable to desoxycorticosterone acetate in a bioassay based on the method of Dorfman.<sup>3</sup> Column chromatography of these extracts suggested that the biologically active material was more polar than desoxycorticosterone.<sup>4</sup>

Paper chromatography by the methods of Burton, Zaffaroni, and Keutmann<sup>5</sup> and of Bush<sup>6</sup> led to the separation of the active material from known biologically potent corticosteroids.<sup>7</sup> A modification of the bioassay in adrenalectomized rats<sup>8</sup> allowed more precise detection of active material and quantitative recovery of the activity from whole urine extracts in one fraction having the chromatographic mobility of aldosterone.<sup>9</sup> Improved methods of extracting urine<sup>10</sup> yielded larger amounts of the active material, from which aldosterone has been crystallized.<sup>11</sup>

Simpson and Tait and their co-workers reported studies<sup>12</sup> of a similar material in bovine adrenal extract and, later, in hog adrenal extract and in adrenal venous blood from the dog and the monkey.<sup>13</sup> In collaboration with Reichstein and Wettstein and their co-workers<sup>14</sup> this steroid was identified as the 18-aldehyde derivative of corticosterone, and was given the definitive name aldosterone. Aldosterone is reported to exist in aqueous solution mainly in the form of the 11,18-cyclohemiacetal.

Other workers have also isolated the active material from adrenal extract.<sup>15</sup>

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and two additional research teams have independently obtained crystalline aldosterone from the adrenal.<sup>16</sup> Farrell and his collaborators<sup>17</sup> have isolated aldosterone from adrenal venous blood of hypophysectomized dogs.

Luetscher, Neher, and Wettstein have reported a sample of the active steroid from the urine of a child with the nephrotic syndrome to be identical with authentic aldosterone in crystalline form, melting point, mixed melting point, and infrared spectrum.<sup>11</sup> Chromatographic and bioassay properties of the free alcohol and the derived mono- and di-acetates, and the products of periodate oxidation were also identical with authentic aldosterone.

The potency of aldosterone can be shown to be many times greater or less than the classical adrenocortical hormones, depending on the kind of activity measured.<sup>18</sup> In the water-loaded adrenalectomized rat, for example, aldosterone and hydrocortisone have opposite effects on sodium excretion.<sup>8</sup> Although the qualitative effects of aldosterone are reproduced by 11-desoxycorticosterone and its 17-hydroxy analogue<sup>8</sup> and by a few synthetic steroids,<sup>19</sup> under these conditions, aldosterone has a considerably greater effect on sodium and potassium excretion than any other compound tested.

Increased amounts of aldosterone have been found in urine extracts from edematous patients with a variety of cardiac and renal diseases, hepatic cirrhosis, and toxemia of pregnancy. Using similar methods of bioassay, Singer and Venning and their co-workers have reported increased excretion of the sodium-retaining corticoid in toxemia of pregnancy,<sup>20</sup> congestive heart failure,<sup>21</sup> and nephrosis.<sup>22</sup> Similar observations in cirrhosis were made by other investigators<sup>23, 24</sup> and in pre-eclamptic toxemia by Chart, Shipley, and Gordon.<sup>25, 26</sup>

The quantity of aldosterone obtained from urine, as with other urinary steroids,<sup>27</sup> can be increased by certain methods of extraction.<sup>10</sup> Neutral extracts contain very little aldosterone.<sup>28</sup> When the urine is immediately extracted at pH 1.0, detectable amounts of activity are obtained from edematous patients, while extracts from normal children and adults are inert or may cause increased sodium excretion in the bioassay. After standing for 24 hours at pH 1.0, appreciable amounts of aldosterone can be extracted from normal urine. It can be demonstrated that extraction within 40 minutes after acidification to pH 1.0 yields approximately the same quantity of hormone as incubation at pH 4.8 for 12 to 24 hours, while somewhat larger amounts can be extracted after hydrolysis with  $\beta$ -glucuronidase.<sup>10</sup> The largest yields are obtained after standing at pH 1.0 for 24 hours. With this technique, the normal urinary output of aldosterone is in the range of 2 to 4  $\mu$ g. in 24 hours. The normal circulating level of aldosterone in human peripheral blood has been estimated by Simpson and Tait<sup>29</sup> at 0.4 to 1.0  $\mu$ g. per liter.

The output of aldosterone is increased during dietary sodium restriction in normal men.<sup>23</sup> This increase corresponds to the reduction in urine sodium excretion during the low sodium intake, and returns to normal levels as sodium output increases after resumption of a normal diet. These reciprocal changes in output of aldosterone and sodium in the urine are not accompanied by significant alteration of glomerular filtration rate or urinary 17-ketosteroids or 17-hydroxycorticoids.<sup>30</sup> On the other hand, the aldosterone output was not increased by a single injection of ACTH gel sufficient to increase five-fold the



output of 17-ketosteroids and 17-hydroxycorticoids. The inverse relationship of urinary sodium excretion and aldosterone output is also seen in patients accumulating edema and during subsequent diuresis, either spontaneous or induced, for example by ACTH.<sup>31, 32</sup> Patients with Addison's disease or adrenalectomy did not excrete aldosterone, but two patients with hypopituitarism had normal urine levels of aldosterone,<sup>33</sup> also suggesting that the secretion of aldosterone is not dependent on any hormone of the anterior pituitary.

Much more information regarding secretion, destruction, and excretion of aldosterone is needed before the role of this hormone is completely established. When aldosterone is administered to patients, retention of sodium and water ensues, while increased amounts of aldosterone appear in urine.<sup>34, 35, 36</sup> Increased excretion of aldosterone in urine is well correlated with sodium retention and accumulation of edema in disease. In normal men, sodium deprivation increases the output of hormone and reduces urine sodium. These observations suggest that aldosterone output reflects a normal mechanism for the control of sodium balance, which is stimulated in certain phases of disease to cause increased sodium retention and edema.

### References

1. LUETSCHER, J. A., JR., A. D. HALL & V. L. KREMER. 1950. *J. Clin. Invest.* **29**: 896.
2. DEMING, Q. B. & J. A. LUETSCHER, JR. 1950. *Proc. Soc. Exptl. Biol. Med.* **73**: 171.
3. DORFMAN, R. I., A. M. POTTS & M. L. FELL. 1947. *Endocrinology*. **41**: 454.
4. HYMAN, E. S. Quoted by J. A. LUETSCHER, JR., Q. B. DEMING & B. B. JOHNSON. 1951. (Jan. 8-10.) *In* Colloquium on the Effects of Steroids on Local and General Water Distribution. Ciba Found. Colloquia on Endocrinol. Vol. **4**. J. & A. Churchill. London, England.
5. BURTON, R. B., A. ZAFFARONI & E. H. KEUTMANN. 1951. *J. Biol. Chem.* **188**: 763.
6. BUSH, I. W. 1952. *Biochem. J.* **50**: 370.
- 7a. LUETSCHER, J. A., JR. & B. B. JOHNSON. 1953. *Am. J. Med.* **15**: 417.
- 7b. LUETSCHER, J. A., JR. & B. B. JOHNSON. 1953. *J. Clin. Invest.* **32**: 585.
8. JOHNSON, B. B. 1954. *Endocrinology*. **54**: 196.
9. LUETSCHER, J. A., JR. & B. B. JOHNSON. 1954. *J. Clin. Invest.* **33**: 276.
10. AXELRAD, B. J., J. E. CATES, B. B. JOHNSON & J. A. LUETSCHER, JR. 1955. *Brit. Med. J.* **1**: 196.
11. LUETSCHER, J. A., JR., R. NEHER & A. WETTSTEIN. 1954. *Experientia*. **10**: 456.
12. GRUNDY, H. M., S. A. SIMPSON & J. F. TAIT. 1952. *Nature*. **169**: 795.
13. SIMPSON, S. A., J. F. TAIT & I. E. BUSH. 1953. *Lancet*. **263**: 226.
- 14a. SIMPSON, S. A., J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW & T. REICHSTEIN. 1953. *Experientia*. **9**: 333.
- 14b. SIMPSON, S. A., J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, O. SCHINDLER & T. REICHSTEIN. 1954. *Experientia*. **10**: 132.
15. KNAUFF, R. E., E. D. NIELSON & W. J. HAINES. 1953. *J. Am. Chem. Soc.* **75**: 4868.
- 16a. MATTOX, V. R., H. L. MASON, A. ALBERT & C. F. CODE. 1953. *J. Am. Chem. Soc.* **75**: 4869.
- 16b. HARMAN, R. E., E. A. HAM, J. J. DEYOUNG, N. G. BRINK & L. H. SARETT. 1954. *J. Am. Chem. Soc.* **76**: 5035.
17. FARRELL, G. L., P. C. ROYCE, E. W. RAUSCHKOLB & H. HIRSCHMANN. 1954. *Proc. Soc. Exptl. Biol. Med.* **87**: 141.
18. GAUNT, R. & A. A. RENZI. 1954. (Nov. 3-4.) *In* A Symposium on Adrenal Function in Infants and Children. : 37. State Univ. New York. Syracuse, N. Y.
19. AXELRAD, B. J., J. E. CATES, B. B. JOHNSON & J. A. LUETSCHER, JR. 1954. *Endocrinology*. **55**: 568.
20. VENNING, E. H., B. SINGER & G. A. SIMPSON. 1954. *Am. J. Obstet. Gynecol.* **67**: 542.
21. SINGER, B. & J. WENER. 1953. *Am. Heart J.* **45**: 795.
22. MCCALL, M. F. & B. SINGER. 1953. *J. Clin. Endocrinol. & Metab.* **13**: 1157.
23. LUETSCHER, J. A., JR. & B. B. JOHNSON. 1954. *J. Clin. Invest.* **33**: 1441.
24. CHART, J. J. & E. S. SHIPLEY. 1953. *J. Clin. Invest.* **32**: 560.
25. CHART, J. J., E. G. SHIPLEY & E. S. GORDON. 1951. *Proc. Soc. Exptl. Biol. Med.* **78**: 244.

26. GORDON, E. S., J. J. CHART, D. HAGEDORN & E. G. SHIPLEY. 1954. *Obstet. & Gynecol.* **4**: 39.
27. VENNING, E. H., I. DYRENFURTH & V. E. KAZMIN. 1953. *Recent Progress in Hormone Research*. Vol. **8**. Academic Press. New York, N. Y.
28. COPE, C. L. & J. GARCIA-LLAURADO. 1954. *Brit. Med. J.* **1**: 1290.
29. SIMPSON, S. A. & J. F. TAIT. 1954. *Ciba Found. Colloquia on Endocrinol.* Vol. **8**. J. & A. Churchill. London, England.
30. LUETSCHER, J. A., JR. & B. J. AXELRAD. 1955. *Proc. Soc. Exptl. Biol. Med.* In press.
31. LUETSCHER, J. A., JR. & O. B. DEMING. 1950. *J. Clin. Invest.* **29**: 1576.
32. LUETSCHER, J. A., JR., O. B. DEMING & B. B. JOHNSON. 1951. *J. Clin. Invest.* **30**: 1530.
33. LUETSCHER, J. A., JR. & B. J. AXELRAD. 1954. *J. Clin. Endocrinol. & Metab.* **14**: 1086.
34. PRUNTY, F. T. G., R. R. McSWINEY, I. H. MILLS & M. A. SMITH. 1954. *Lancet*. **2**: 620.
35. WARD, L. E., H. F. POLLEY, C. H. SLOCUMB, P. S. HENCH, H. L. MASON, V. R. MATTOX & M. H. POWER. 1954. *Proc. Staff Meetings Mayo Clinic*. **29**: 649.
36. CURTIS, R. H. & J. A. LUETSCHER, JR. Unpublished results.

# COMPARATIVE ACTION OF ALDOSTERONE AND 9-ALPHA-FLUOROHYDROCORTISONE IN MAN\*

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Recently, two crystalline adrenal steroids of high biological activity have become available for clinical investigation. The first, aldosterone, is a naturally occurring steroid which has been isolated from adrenal cortical extracts.<sup>1-4</sup>

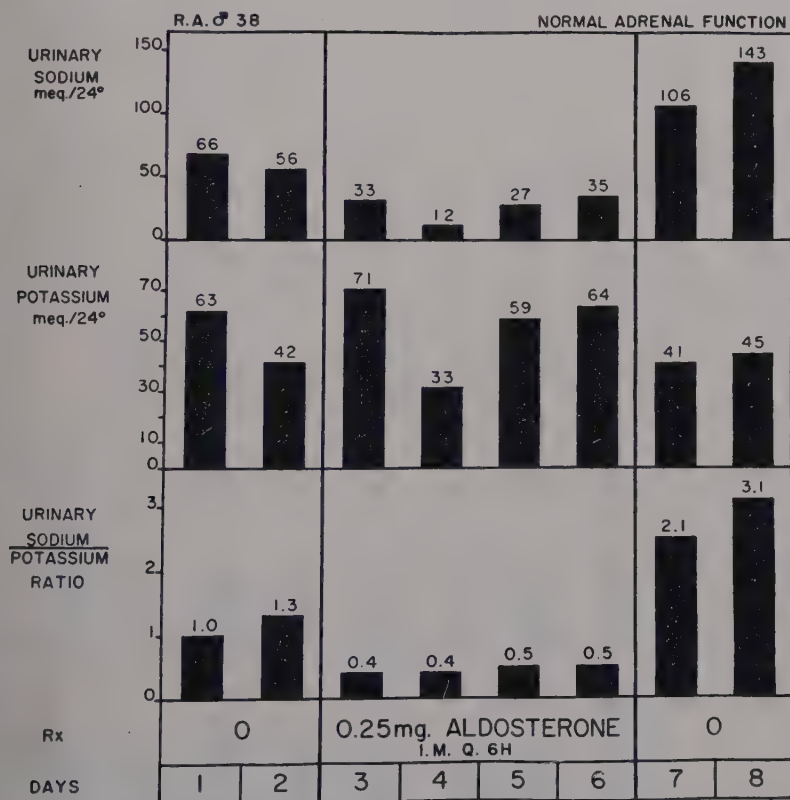


FIGURE 1. Effects of aldosterone on urinary excretion of sodium and potassium in a patient with normal adrenal function maintained on a constant diet (Na = 86 mEq., K = 77 mEq.).

The second, 9 $\alpha$ -fluorohydrocortisone, is a synthetic analog of hydrocortisone.<sup>5-8</sup> The quantities of aldosterone available for clinical investigation at present are

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extremely small, whereas the supply of synthetically prepared 9 $\alpha$ -fluorohydrocortisone is quite adequate. The present studies were designed to evaluate the potency of these two steroids in man with respect to their inorganic and organic metabolic-regulating activity, as well as their relative capacity to inhibit pituitary ACTH secretion. Because of the relatively small quantities of aldosterone available, the observations must be considered to be provisional. These studies have been carried out in the Metabolic Ward of the Peter Bent Brigham Hospital, Boston, Mass.

### 1. Aldosterone

(a) *Studies on subject R. A. with intact adrenals.* Metabolic observations were made on a patient with normal adrenal function who was given aldosterone in aqueous solution, intramuscularly, 0.25 mg. every six hours (one mg. daily) for four successive days. The principal changes observed were sodium and chloride retention and a marked decrease in urinary Na/K ratio (FIGURE 1). Potassium excretion was altered less significantly. There was no significant change in the urinary excretion of total nitrogen, inorganic phosphorus, uric acid, or true urinary glucose<sup>9</sup> (FIGURE 2). An appreciable decrease in the level of circulating eosinophils<sup>10</sup> was noted. No significant reduction in the urinary

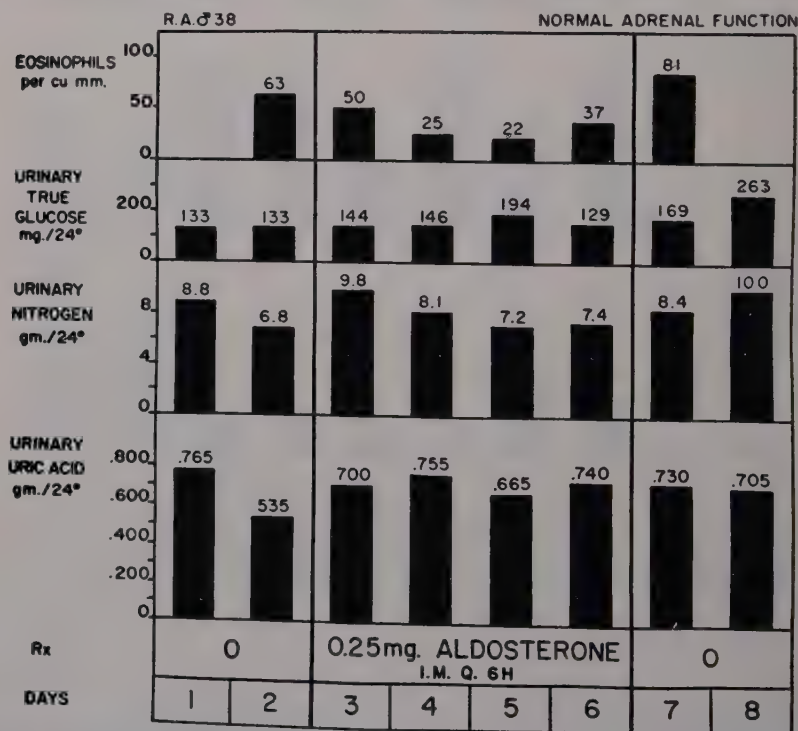


FIGURE 2. Effect of aldosterone on organic metabolism in a patient with normal adrenal function maintained on a constant diet (Na = 86 mEq., K = 77 mEq., protein = 62 gm., carbohydrate = 227 gm., fat = 80 gm.).



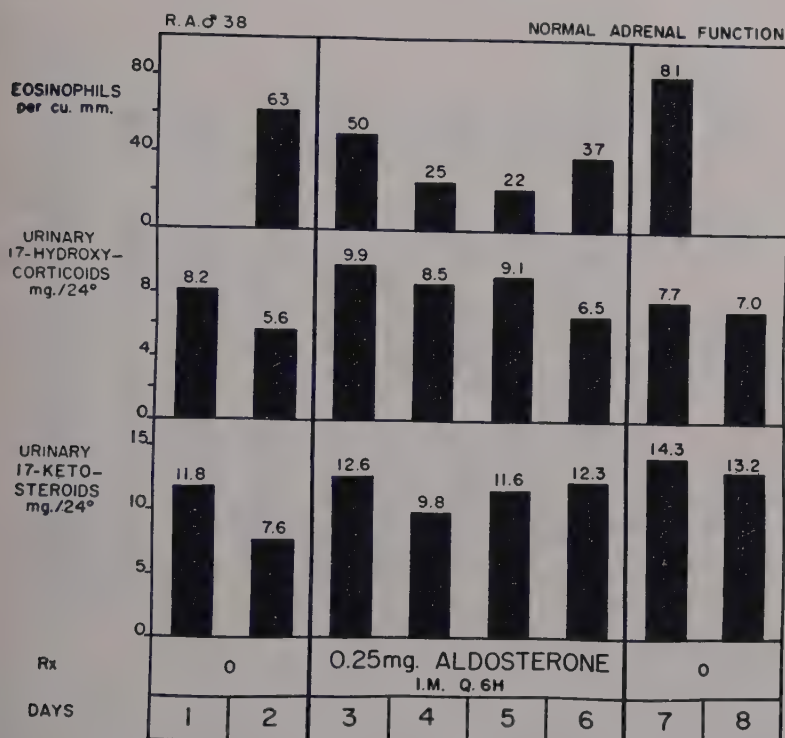


FIGURE 3. Effects of aldosterone on urinary excretion of endogenous steroids in a patient with normal adrenal function.

excretion of total 17-hydroxycorticoids<sup>11</sup> and 17-ketosteroids<sup>12, 13</sup> occurred (FIGURE 3). The failure to observe an appreciable reduction in urinary steroid excretion suggests that aldosterone, in the dosage employed, is not an effective inhibitor of pituitary ACTH secretion in man.

(b) *Studies on patient W. G. with classical Addison's disease.* A patient with Addison's disease was given aldosterone, 0.25 mg. every six hours (one mg. daily) intramuscularly over a period of six days. As was observed in the subject with intact adrenals, aldosterone exerted its most striking effect on the urinary excretion of sodium (FIGURE 4). The Na/K ratio decreased from 2.1 during the control period on cortisone (25 mg. daily) to 0.5 and 0.4 during the administration of aldosterone. With continued aldosterone therapy, the Na/K ratio approached a value of 1.0 on the sixth day. It is of some interest to note that, on this dose of aldosterone, the patient gained a total of 3.5 kilograms in weight in six days and presented every evidence of accumulating excessive sodium and water. On the first day of aldosterone therapy, there was observed a small increase in the urinary excretion of potassium (22 per cent) and total nitrogen (6 per cent) without an appreciable alteration in the values for uric acid, inorganic phosphorus, and true glucose (FIGURE 5). The tolerance

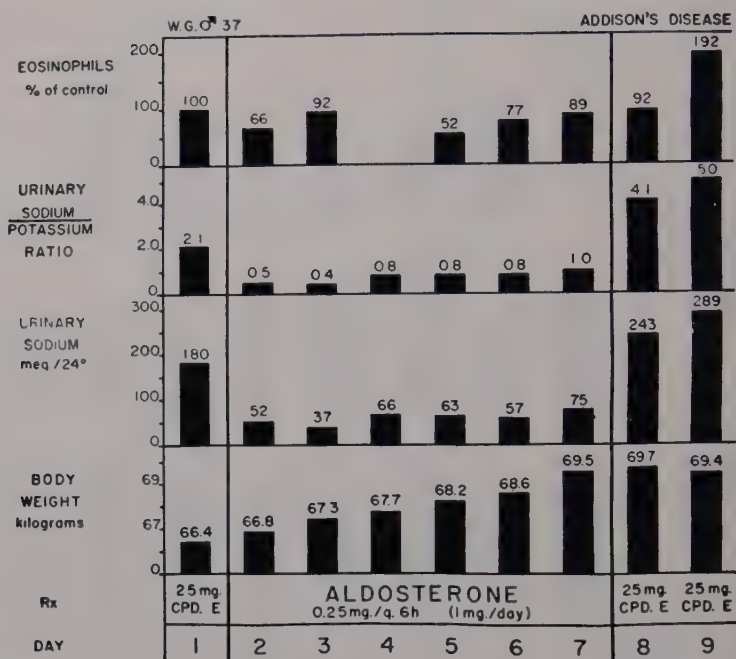


FIGURE 4. Effect of aldosterone in a patient with Addison's disease maintained on a constant diet ( $\text{Na} = 184 \text{ mEq.}$ ,  $\text{K} = 97 \text{ mEq.}$ ).

for intravenously administered glucose was not altered (FIGURE 6). There appeared to be a small but definite decrease in the level of circulating eosinophils in conjunction with the aldosterone therapy, the average decrease during the six days of treatment being 28 per cent. The significance of this decrease is enhanced by the pronounced rebound eosinophilia (92 per cent increase over base line) which occurred on the second day following the discontinuance of aldosterone and resumption of control cortisone therapy, *i.e.* 25 mg. daily by mouth. Since it has been suggested that aldosterone might exert a highly specific effect on pigmentation in Addison's disease,<sup>3</sup> careful studies employing the principle of reflectance spectrophotometry<sup>14</sup> were made. As may be seen (FIGURE 7), no decrease in the melanin-absorption spectrum was noted during the six days of aldosterone therapy.

The effectiveness of several routes of administration of aldosterone was also studied in patient W. G. These included the intravenous administration of one mg. of the hormone over a period of eight hours, the oral and intramuscular administration of the same quantity of aldosterone as a single dose, and the intramuscular administration of the same total quantity of the hormone but given in divided doses, *i.e.*, 0.25 mg. every six hours (FIGURE 8). It was readily apparent that the most efficient method of administration was to give the hormone intramuscularly in divided doses (every six hours). The most intense metabolic effect occurred in conjunction with the intravenous infusion of hormone.

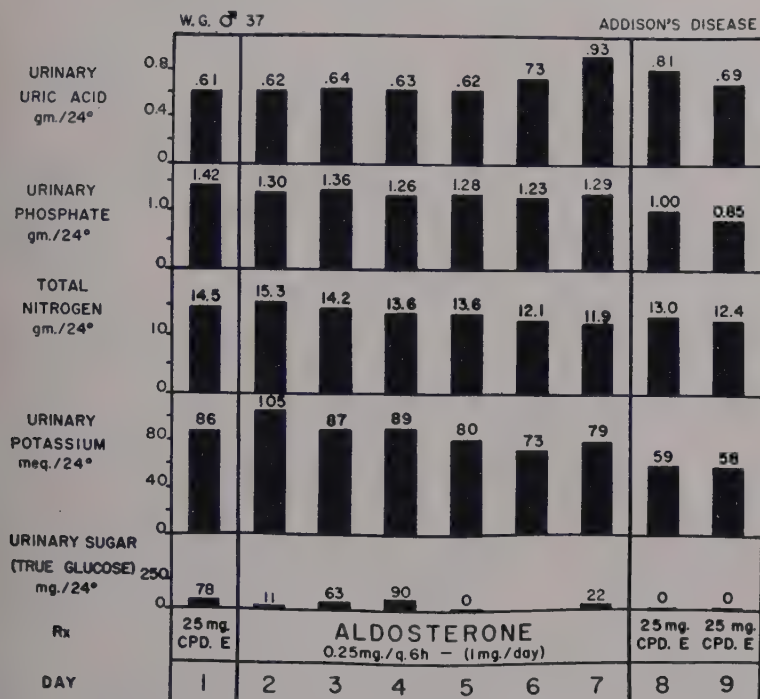


FIGURE 5. Effect of aldosterone on organic metabolism in a patient with Addison's disease maintained on a constant diet (Na = 184 mEq., K = 97 mEq., carbohydrate = 355 gm., protein = 101 gm., fat = 136 gm.).

## 2. Aldosterone versus 9 $\alpha$ -Fluorohydrocortisone

(a) *Studies on patient W. G. (Addison's disease).* Patient W. G. was given 200 micrograms of aldosterone intravenously over a period of four hours while being maintained on a constant regimen. On the following day, during a corresponding period, 200 micrograms of 9 $\alpha$ -fluorohydrocortisone was administered in a similar fashion (FIGURE 9). During the period of infusion there occurred, in both instances, a rapid and profound decrease in sodium excretion and an appreciable increase in potassium excretion. The magnitude and rate of change were approximately the same for the two compounds. Following the discontinuance of the intravenous infusion, however, the escape from aldosterone became apparent at the end of two hours and was virtually complete in approximately six hours, whereas fluorohydrocortisone continued to exhibit a near maximal physiological effect at the end of six hours. This experiment suggested that the biological activity of the two substances was inherently the same, but that fluorohydrocortisone exerted a more prolonged period of effective action—possibly dependent on a slower rate of degradation or inactivation.

The effect of 1 mg. of aldosterone administered as a single dose intramuscularly (both aqueous solution and solution in oil) was compared to the metabolic effect of 250 micrograms of 9 $\alpha$ -fluorohydrocortisone given orally once

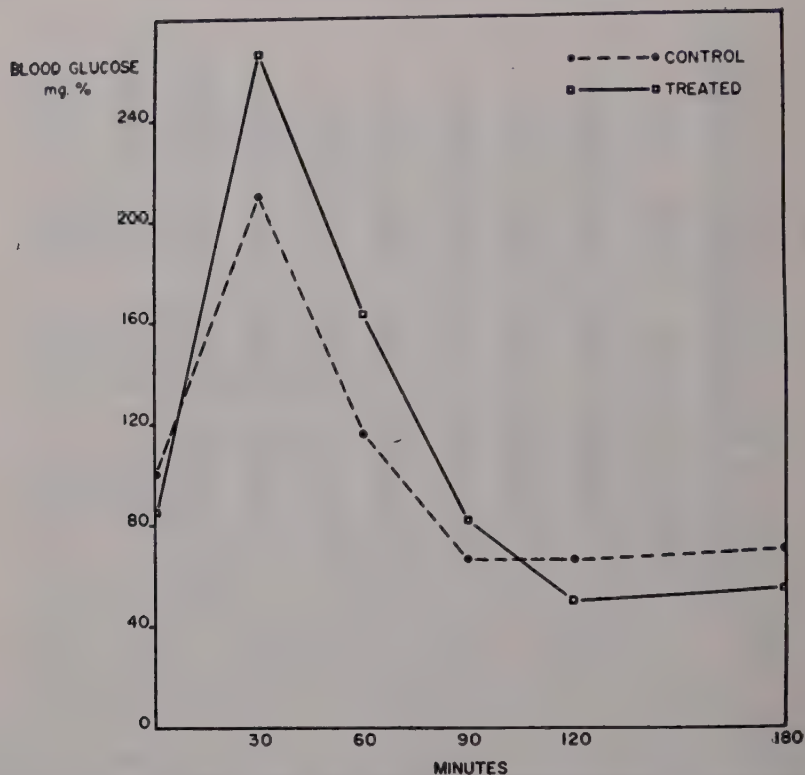


FIGURE 6. Intravenous glucose tolerance test before and on the fifth day of aldosterone administration (1 mg. q.d.). Amount of glucose infused: 0.5 gm. per kilogram over 30 minutes.

daily at approximately 8 AM (FIGURE 10). It is readily apparent that 1 mg. of aldosterone administered in the manner described was approximately equivalent in electrolyte regulating effect to a single dose of 250 micrograms of fluorohydrocortisone given once daily by mouth. There was indication too that the level of circulating eosinophils rose during the day 1 mg. of aldosterone was substituted for the 250 micrograms of fluorohydrocortisone.

(b) *Studies on patients W. L. and J. C. (Addison's disease).* The relative effectiveness of 1 mg. of aldosterone, 9 $\alpha$ -fluorohydrocortisone, 9 $\alpha$ -fluorocorticosterone and hydrocortisone was studied in two additional patients with Addison's disease. In all instances, the compounds were given intravenously over a period of eight hours (FIGURE 11). Whereas hydrocortisone was ineffective at this dose level, as might have been anticipated, aldosterone and the two fluorinated compounds exerted a marked sodium retaining effect of approximately the same degree. The duration of the aldosterone effect, however, was less than that of either fluorohydrocortisone or fluorocorticosterone. At this dose level, the eosinopenia produced by aldosterone was comparable to that produced by fluorohydrocortisone, whereas no eosinophil fall resulted from the fluorocorticosterone infusion.



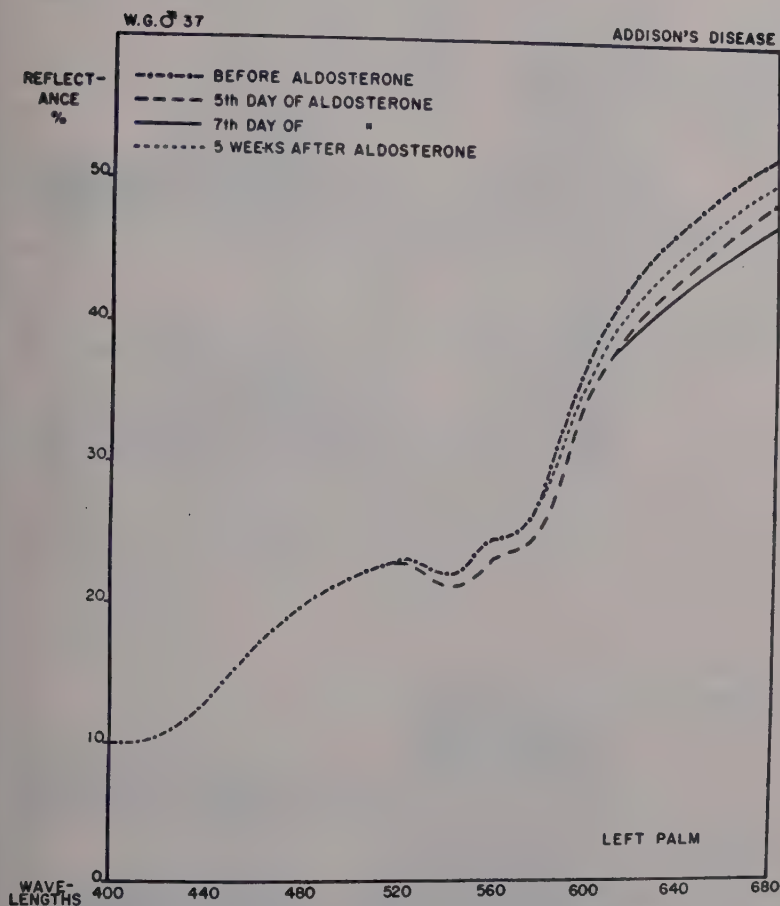


FIGURE 7. The effects of aldosterone (0.25 mg. q. 6 hr.) on pigmentation of the skin. Per cent reflectance of incident light (abscissa) at different wavelengths (ordinate). Lightening of the skin would produce an upward deflection of the curve.

(c) *Studies on subject R. A. with intact adrenals.* The substitution of 1 mg. of aldosterone administered orally once daily for an equivalent quantity of  $\alpha$ -fluorohydrocortisone similarly administered permitted a marked escape in sodium retention and a consequent increase in the urinary Na/K ratio (FIGURE 2). Simultaneously, a rise in the level of circulating eosinophils was noted, and a decrease in pituitary inhibition was suggested by a return toward normal of the urinary excretion of 17-hydroxycorticoids and 17-ketosteroids during aldosterone administration. The effects of aldosterone and fluorohydrocortisone on the urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids are further compared in FIGURE 13. It is apparent that aldosterone produced little change, if any, in the excretion of endogenous steroids, whereas the same daily dosage of fluorohydrocortisone produced a marked drop in the excretion

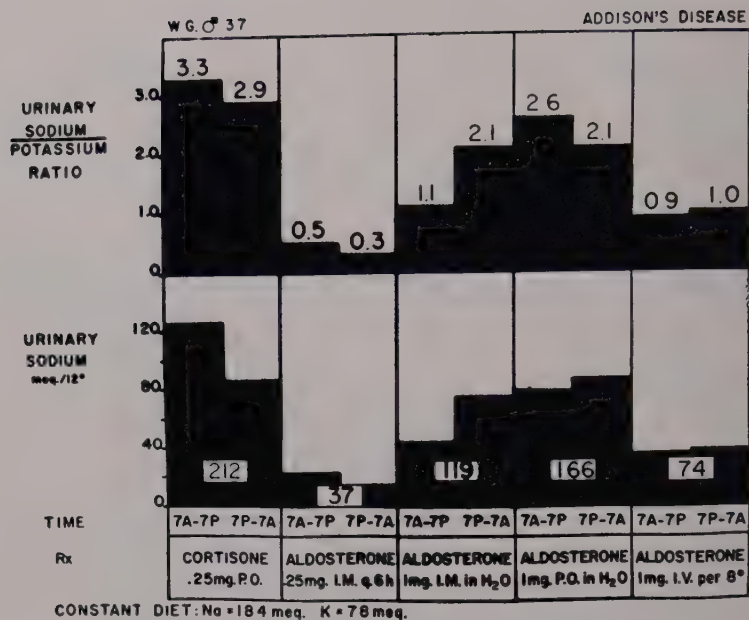


FIGURE 8. Studies on aldosterone: routes of administration.

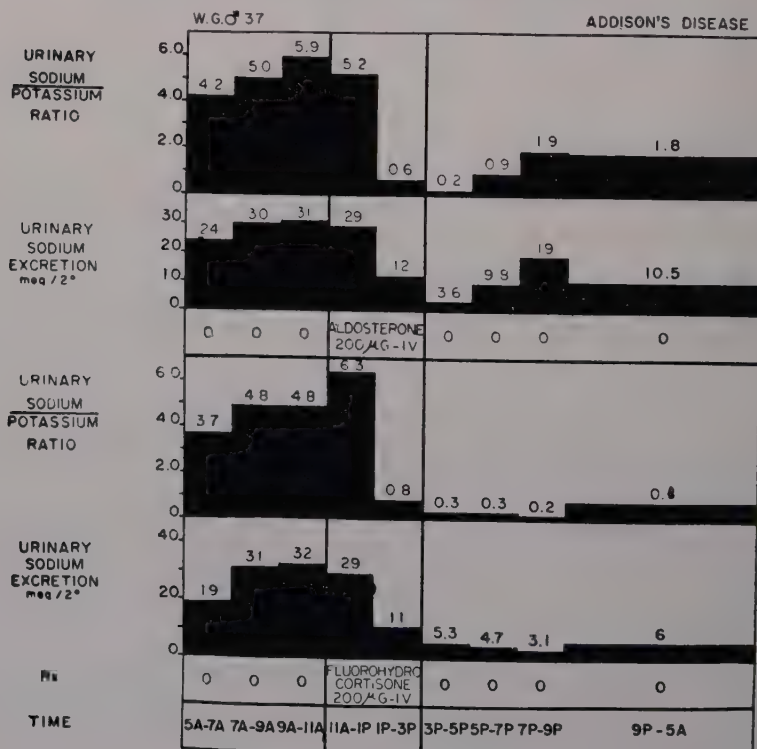


FIGURE 9. Comparative effectiveness of intravenous aldosterone and fluorohydrocortisone.

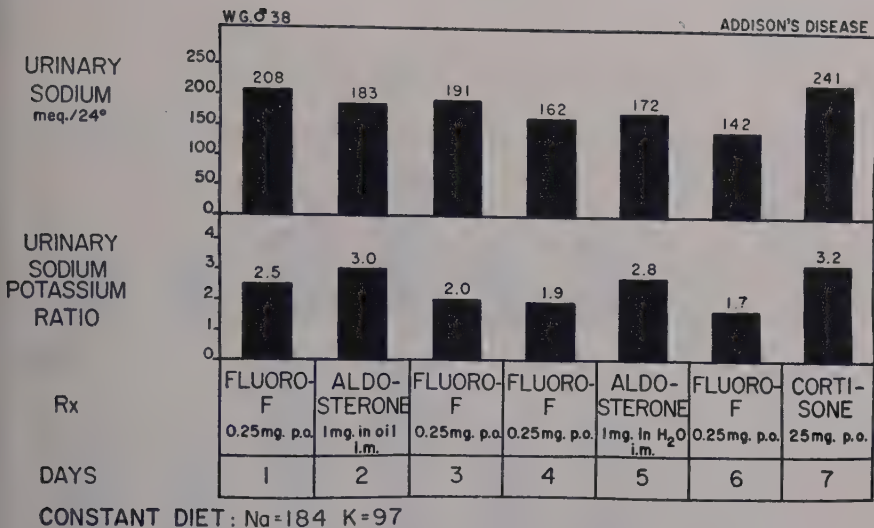


FIGURE 10. Comparative effects of aldosterone and fluorohydrocortisone in a patient with Addison's disease.

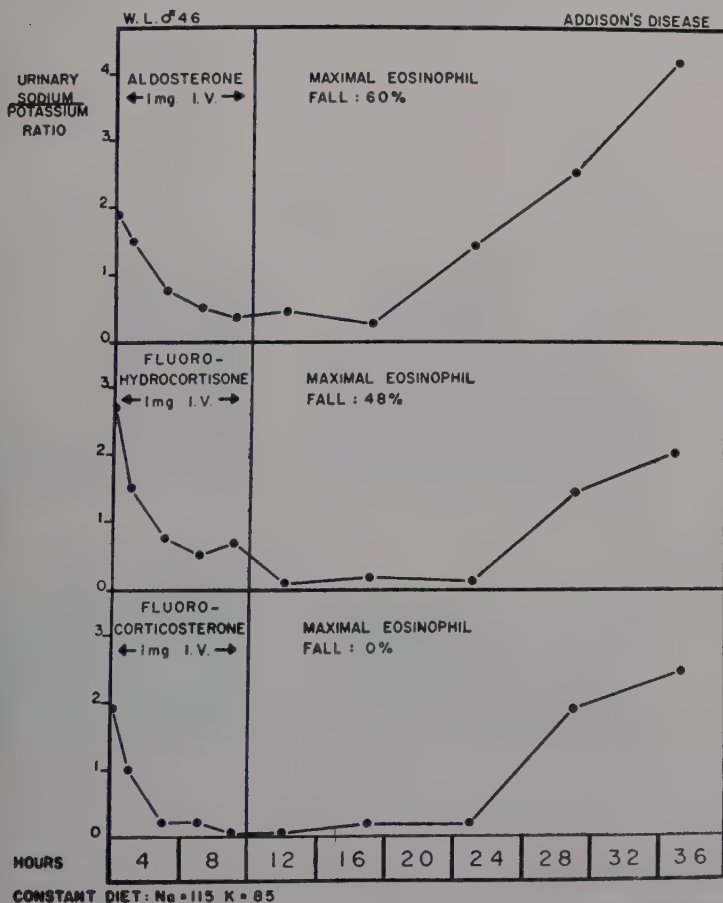


FIGURE 11. Relative effectiveness of intravenous aldosterone, 9α-fluorohydrocortisone and 9α-fluorocorticosterone on urinary excretion of sodium and potassium in a patient with Addison's disease maintained on a constant diet. One mg. of hydrocortisone similarly administered failed to produce significant change in the urinary Na/K ratio.

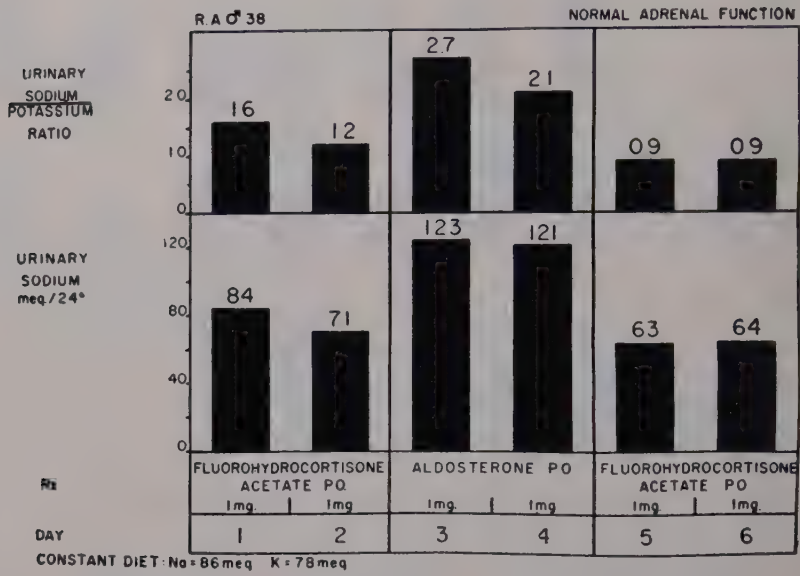


FIGURE 12. Substitution of aldosterone (1 mg. P.O.) for fluorohydrocortisone acetate (1 mg. P.O.).

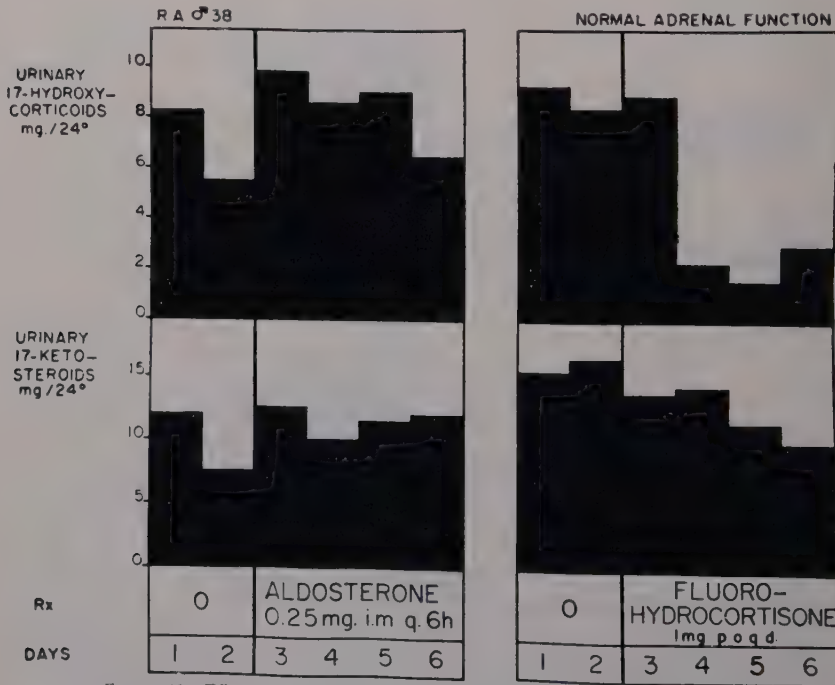


FIGURE 13. Effects of aldosterone and fluorohydrocortisone on steroid excretion.



of 17-hydroxycorticoids and a suggestive decrease in the excretion of 17-ketosteroids.

### Summary

These studies suggest that, in man, aldosterone exerts an important influence on mineral metabolism and a small but definite influence on the level of circulating eosinophils. At the dose level of one mg. per day, no other influence on organic metabolic processes and no definite pituitary inhibiting action could be demonstrated. Using divided doses given intramuscularly as an aqueous solution, it was possible to induce excessive sodium and chloride retention over the short period of study permitted by the restricted quantity of hormone presently available. In its sodium-retaining effect, one mg. of aldosterone given in four divided doses intramuscularly every six hours is equivalent to one mg. of 9 $\alpha$ -fluorohydrocortisone given orally as a single daily dose. In a classical case of Addison's disease, no amelioration of melanin pigmentation followed six days of therapy with aldosterone (one mg. daily) when measured by the technic of reflectance spectrophotometry. This observation is all the more significant since, during this six-day period, excessive sodium and water retention occurred, as indicated by a gain in body weight of 3.5 kilograms.

### References

1. SIMPSON, S. A., J. F. TAIT, A. WETTSTEIN, R. NEHER, J. V. EUW & T. REICHSTEIN. 1953. Isolierung eines neuen kristallisierten Hormons aus Nebennieren mit besonders hoher Wirksamkeit auf den Mineralstoffwechsel. *Experientia*. **9**: 333-335.
2. SIMPSON, S. A., J. F. TAIT, A. WETTSTEIN, R. NEHER, J. V. EUW, O. SCHINDLER & T. REICHSTEIN. 1954. Die Konstitution des Aldosterons. Über Bestandteile der Nebennierenrinde und verwandte Stoffe. *Helv. Chim. Acta*. **37**: 1200-1223.
3. MACH, R. S., J. FABRE, A. DUCKERT, R. BORTH & P. DUCOMMUN. 1954. Action clinique et métabolique de l'aldosterone (electrocortine). *J. suisse med.* **84**: 407-416.
4. GAUNT, R., A. A. RENZI & J. J. CHART. Aldosterone—A review. Gordon Research Conference Lecture. To be published.
5. FRIED, J. & E. F. SABO. 1954. 9 $\alpha$ -fluoro derivatives of cortisone and hydrocortisone. *J. Am. Chem. Soc.* **76**: 1455.
6. FRIED, J. 1955. Biological effects of fluorohydrocortisone and related halogenated steroids in animals. *Ann. N. Y. Acad. Sci.* **61** (2): 573-581.
7. THORN, G. W., J. C. LAIDLAW & A. GOLDFIEN. 1955. Studies on the sodium-retaining effect of adrenocortical steroids. *Ciba Colloquia on Endocrinology*. **8**: 343-360.
8. GOLDFIEN, A., J. C. LAIDLAW, N. ABU HAYDAR, A. E. RENOLD & G. W. THORN. 1955. Fluorohydrocortisone and chlorohydrocortisone, highly potent derivatives of compound F. *New Engl. J. Med.* **252**: 415-421.
9. RENOLD, A. E. & E. R. FROESCH. In preparation.
10. FISHER, B. E. & E. R. FISHER. 1951. Observations on the eosinophil count in man, a proposed test of adrenal cortical function. *Am. J. Med. Sci.* **221**: 121-132.
11. REDDY, W. J. 1954. Modification of the Reddy-Jenkins-Thorn method for the estimation of 17-hydroxycorticoids in urine. *Metabolism*. **3**: 489-492.
12. DREKTER, I. J., A. HEISLER, G. R. SCISM, S. STERN, S. PEARSON & T. H. MCGAVACK. 1952. The determination of urinary steroids. I. The preparation of pigment-free extracts and a simplified procedure for the estimation of total 17-ketosteroids. *J. Clin. Endocrinol. & Metab.* **12**: 55-65.
13. HOLTORFF, A. F. & F. C. KOCH. 1940. The colorimetric estimation of 17-ketosteroids and their application to urine extracts. *J. Biol. Chem.* **135**: 377-392.
14. HALL, T. C., B. H. MCCracken & G. W. THORN. 1953. Skin pigmentation in relation to adrenal cortical function. *J. Clin. Endocrinol. & Metab.* **13**: 243-257.

# EFFECTS OF ALDOSTERONE (ELECTROCORTIN), 9 ALPHA-FLUOROHYDROCORTISONE ACETATE, AND 1-DEHYDROCORTISONE (METACORTANDRACIN) IN RHEUMATOID ARTHRITIS

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Since the discovery of the antirheumatic and anti-inflammatory effects of cortisone, many different steroids have been investigated in an effort to find superior compounds for the treatment of rheumatoid arthritis and other diseases whose manifestations are inhibited by cortisone. The physiologic properties other than antirheumatic activity have been studied in certain of these substances also. The finding that slight modifications in the steroid molecule may lead to compounds with markedly different activities is not surprising. Nevertheless, it has not been possible in the past to predict with much accuracy what differences certain changes in structure might produce, a few notable exceptions notwithstanding.

Now, various studies are defining more clearly the structural requirements for steroids with antirheumatic activity. These studies also suggest that certain structural alterations may modify specific physiologic activities of these cortisonelike antirheumatic steroids without diminishing or destroying, but sometimes even enhancing, their desirable antirheumatic capacities.

## *Aldosterone (Electrocortin)*

*Source and structure.* Aldosterone is an adrenocortical hormone which was isolated recently from extracts of whole adrenal glands of cattle and hogs,<sup>1-3</sup> from the amorphous fraction of beef adrenal glands,<sup>4</sup> and from the urine of a nephrotic patient.<sup>5</sup> Aldosterone may be that part of the amorphous fraction which has the greatest effect on the metabolism of electrolytes (hence the provisional name, electrocortin, given to it prior to the identification of its structure).<sup>6</sup>

In chemical structure, aldosterone is closely related to corticosterone, the only difference being that, at C-18, aldosterone has an aldehyde group (hence its name) whereas corticosterone has a methyl group.<sup>7</sup> Unlike cortisone and hydrocortisone, both aldosterone and corticosterone lack the hydroxyl group at C-17 (FIGURE 1).

*Results of use of aldosterone.* Limited supplies of aldosterone have permitted us to administer this steroid to only two patients with rheumatoid arthritis and for only six days each.† The first patient (case 1), a 34-year-old man with severe rheumatoid arthritis, was given aldosterone intramuscularly every six hours for six days in total daily doses of 200, 250, 400, 400, 800, and 280  $\mu$ g. (FIGURE 2). The second patient (case 2), a 37-year-old woman with mild rheumatoid arthritis, was given aldosterone intramuscularly every six hours

\* The Mayo Foundation, Rochester, Minn., is a part of the Graduate School of the University of Minnesota.

† Part of the aldosterone was supplied by Professor Tadeus Reichstein and by Doctor Kenneth W. Thompson, Medical Director of Organon, Inc. of Orange, N. J. and by Spencer M. Fossel; the rest was supplied by our colleagues, Doctor H. L. Mason and Doctor V. R. Mattox.

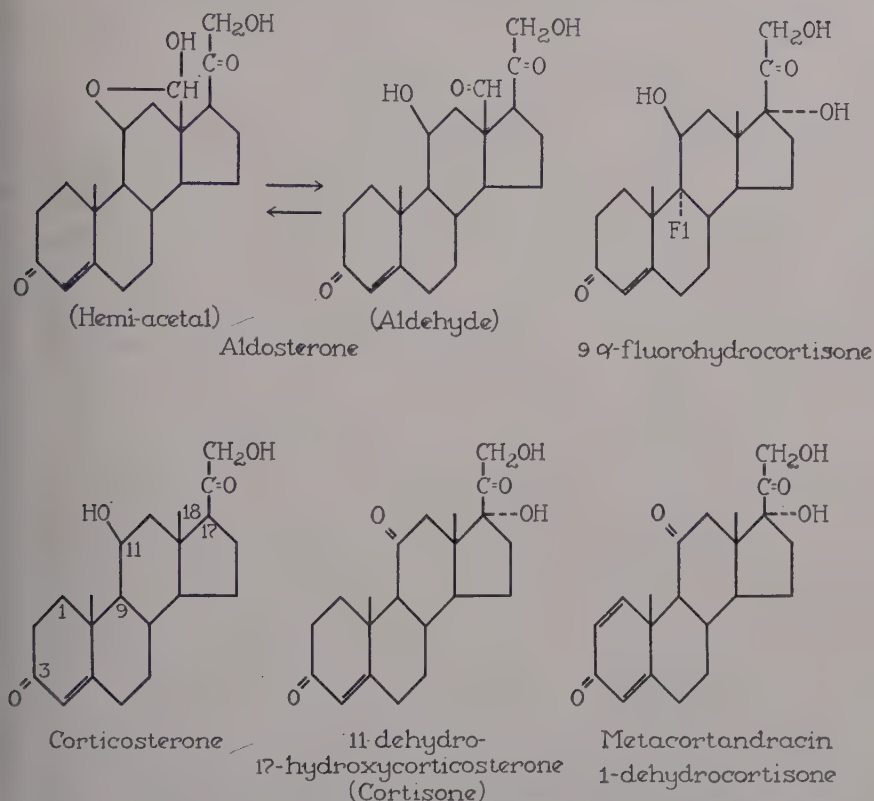


FIGURE 1. Aldosterone exists in two tautomeric forms, the hemi-acetal and the aldehyde, the former apparently being the chief form in which the hormone exists in solution.

for six days in total daily doses of 800  $\mu$ g. for three days, 600  $\mu$ g. for one day, 1,000  $\mu$ g. for one day and 680  $\mu$ g. on the sixth day (FIGURES 3 to 6). No anti-rheumatic effect could be detected in either case, nor did the arthritis become worse. Further investigations of possible antirheumatic activity, however, should be conducted with larger doses of aldosterone. Aldosterone caused retention of fluid in both cases. Balance studies revealed retention of sodium and chloride but no loss of potassium, calcium, inorganic phosphate, or nitrogen during administration of aldosterone to the second patient. Details concerning these two cases have been presented elsewhere.<sup>8</sup>

#### *9 $\alpha$ -Fluorohydrocortisone Acetate*

*9 $\alpha$ -Halogen derivatives of cortisone and hydrocortisone.* The four 9 $\alpha$ -halogen derivatives (fluoro-, chloro-, bromo-, and iodo- derivatives) of hydrocortisone and cortisone have adrenocortical-like activity which was measured by various tests on animals.<sup>9-14</sup> The activity of the different halogenated compounds is inversely proportional to the atomic weight of the substituted halogen when measured by the effect of the compounds on the deposition of glycogen in the

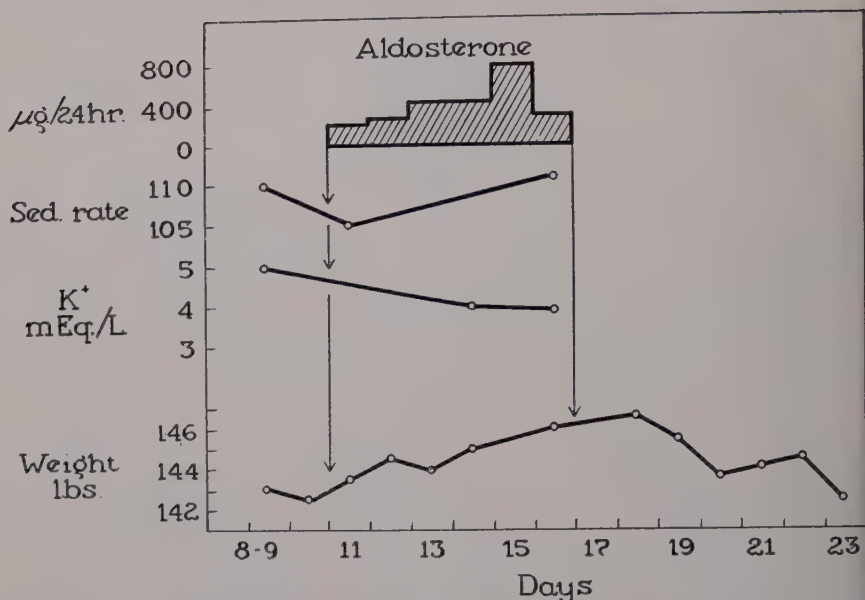


FIGURE 2 (case 1). Effect of aldosterone on sedimentation rate, serum potassium, and body weight.

livers of adrenalectomized rats.<sup>14</sup> The halogenated hydrocortisones are more active than the analogous halogenated cortisones.<sup>9, 10, 13, 14</sup>

*Effects on animals.* 9 $\alpha$ -Fluorohydrocortisone acetate is the most active halogenated steroid known to date. It is more active than desoxycorticosterone in terms of resultant sodium retention in adrenalectomized dogs<sup>13</sup> and more potent than cortisone acetate in causing deposition of glycogen in the livers of adrenalectomized rats<sup>9</sup> and in the production of eosinopenia in adrenalectomized dogs.<sup>13</sup>

*Effects on patients.* In brief treatment of a few patients with Addison's disease, both 9 $\alpha$ -fluorohydrocortisone acetate and 9 $\alpha$ -chlorohydrocortisone acetate have been reported to be much more effective than comparable amounts of hydrocortisone or desoxycorticosterone.<sup>13, 15</sup> Boland and Headley,<sup>16, 17</sup> Bunim,<sup>18</sup> and Bayles<sup>19</sup> recently reported that 9 $\alpha$ -fluorohydrocortisone acetate was antirheumatic.

*Our use and results in rheumatoid arthritis.* Our results from 9 $\alpha$ -fluorohydrocortisone acetate given orally to four patients with rheumatoid arthritis indicate that it has marked antirheumatic effect and even more marked effects on the metabolism of electrolytes and water.\*

One patient (case 2) received 9 $\alpha$ -fluorohydrocortisone acetate in daily doses of 4 mg. for six days, then 6 mg. for six days (FIGURES 3 to 6). Another patient (case 3) received this compound for 26 days in doses up to 4 mg. per day (FIGURES 7 and 8). A third (case 4) was given 9 $\alpha$ -fluorohydrocortisone

\* The 9 $\alpha$ -fluorohydrocortisone acetate was supplied through the courtesy of Doctors Augustus Gibson and Elmer Alpert of Merck and Co., Inc., Rahway, N. J. and Doctor Henry A. Strade, Associate Medical Director of E. R. Squibb and Sons, New Brunswick, N. J.



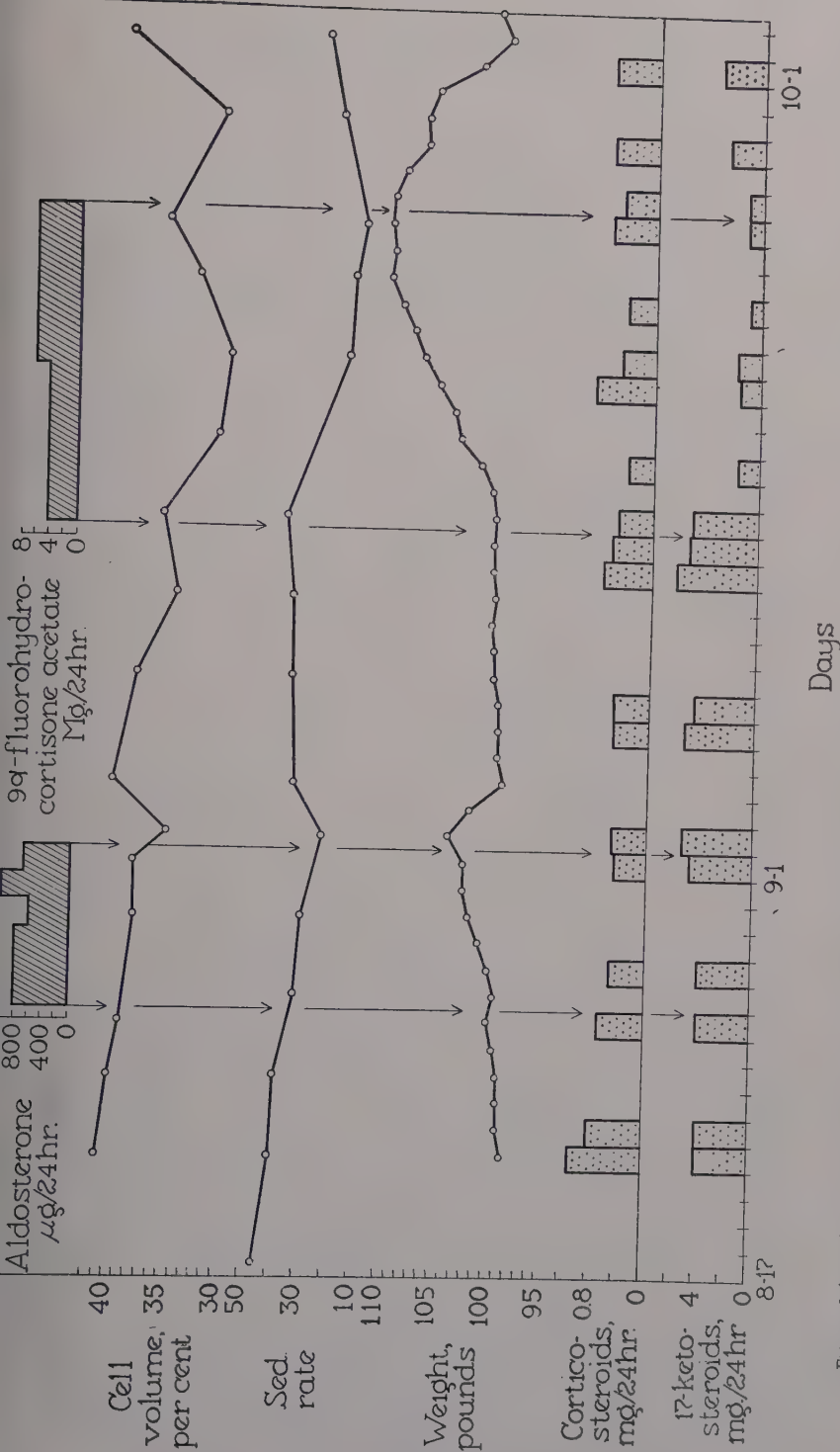


FIGURE 3 (case 2). Effect of aldosterone and 9 $\alpha$ -fluorohydrocortisone acetate on cell volume, sedimentation rate, body weight, and urinary excretion of corticosteroids and 17-ketosteroids.

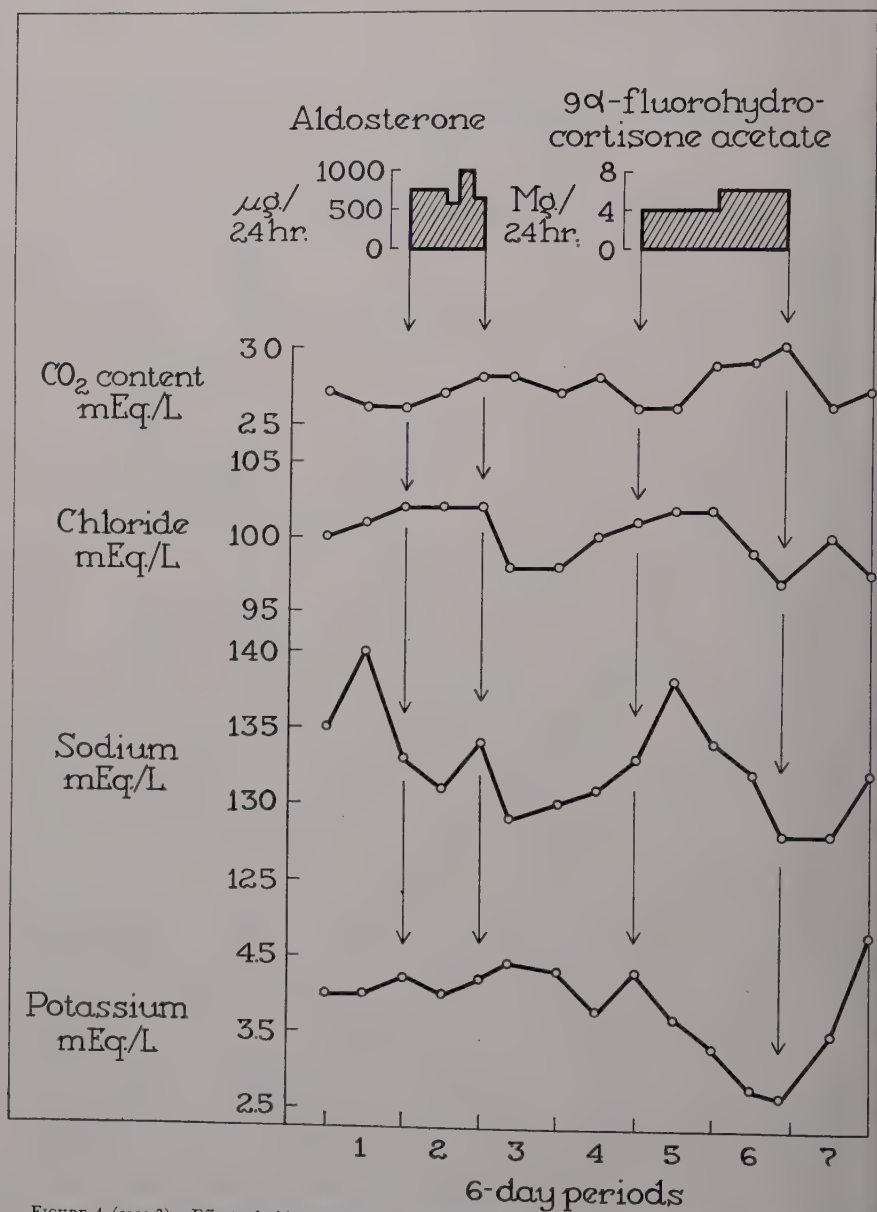


FIGURE 4 (case 2). Effect of aldosterone and 9 $\alpha$ -fluorohydrocortisone acetate on carbon dioxide content, chloride, sodium and potassium of the serum.

acetate for 28 days in doses up to 8 mg. per day (FIGURES 9 and 10). Detailed results in these three cases have been presented previously.<sup>8</sup>

To a fourth patient (case 5), a 52-year-old man with moderately severe rheumatoid arthritis, 9 $\alpha$ -fluorohydrocortisone acetate was administered for 84

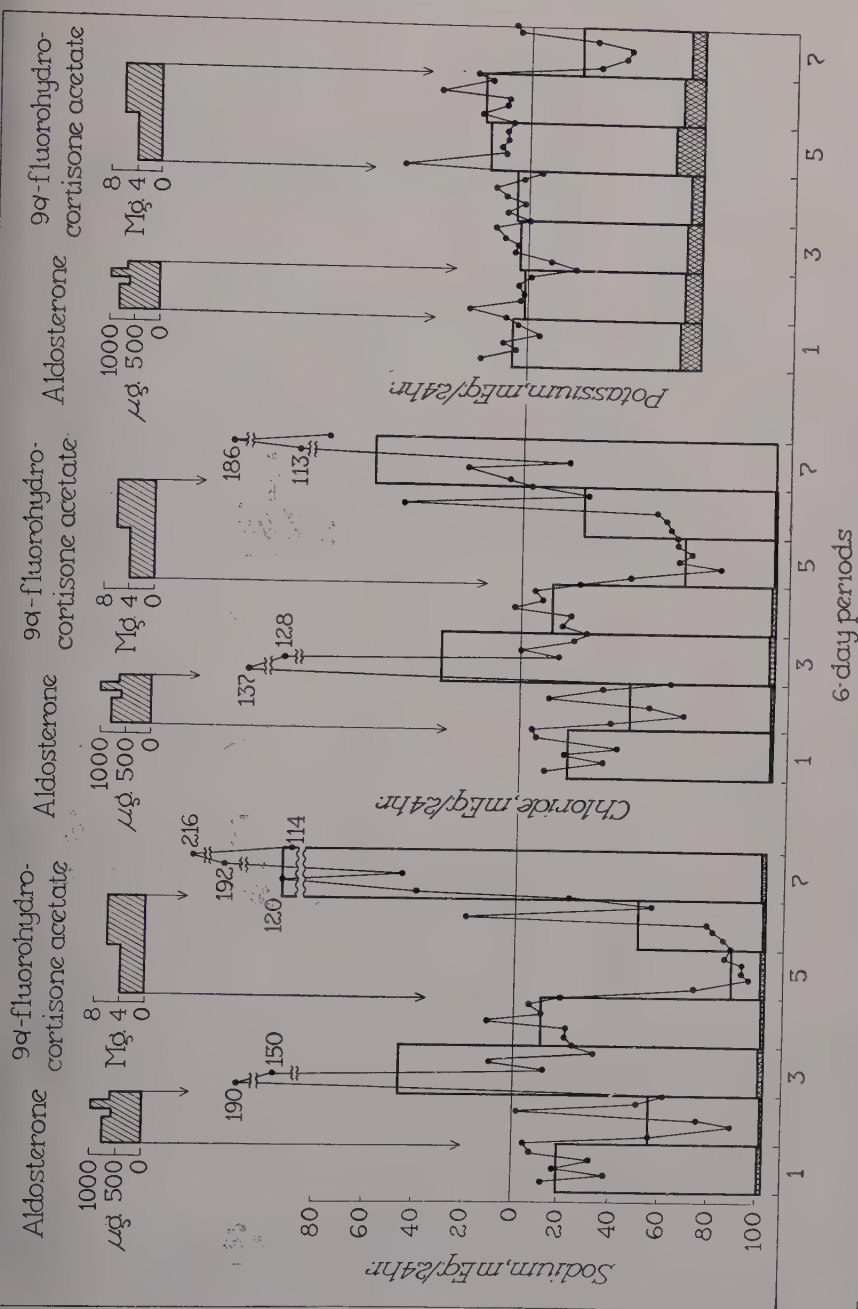


FIGURE 5 (case 2). Effect of aldosterone and 9α-fluorohydrocortisone acetate on balances of sodium, chloride, and potassium. In this figure and in FIGURE 6, daily intake is measured downward (but not blocked in) from the 0 line and is represented by the distance from the 0 line to the bottom line of the column. The average daily excretion (fecal, hatched column; urinary, clear column) is charted upward from the bottom line. Each column represents a six-day period. Each dot represents the daily balance calculated by subtracting the daily urinary excretion plus one sixth of the fecal excretion from the daily intake. A negative balance is indicated by the position of the top of the column or of the dot, above the 0 line; a positive balance, below the 0 line.

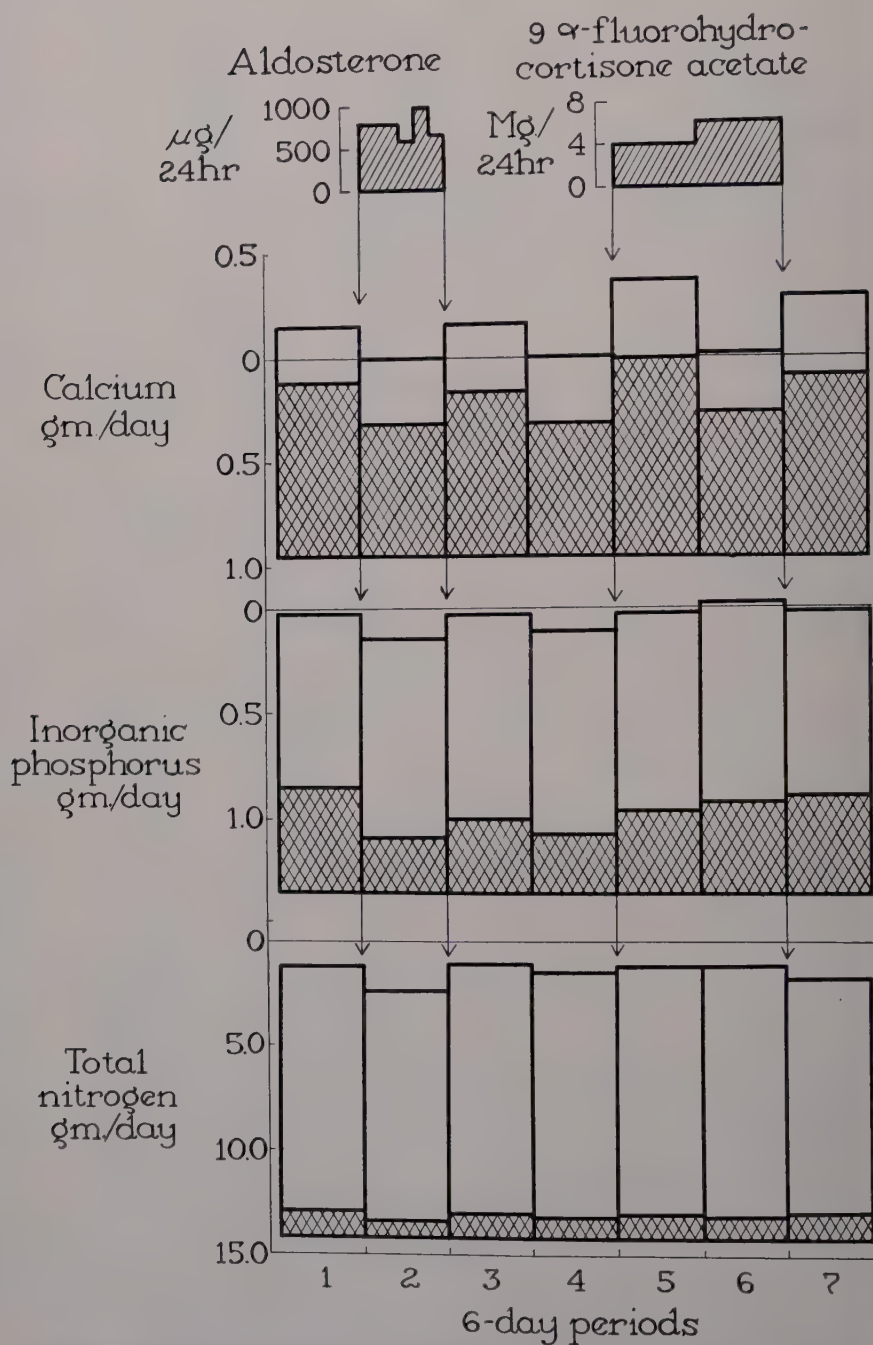


FIGURE 6 (case 2). Effect of aldosterone and 9 $\alpha$ -fluorohydrocortisone acetate on balances of calcium, inorganic phosphorus, and total nitrogen.



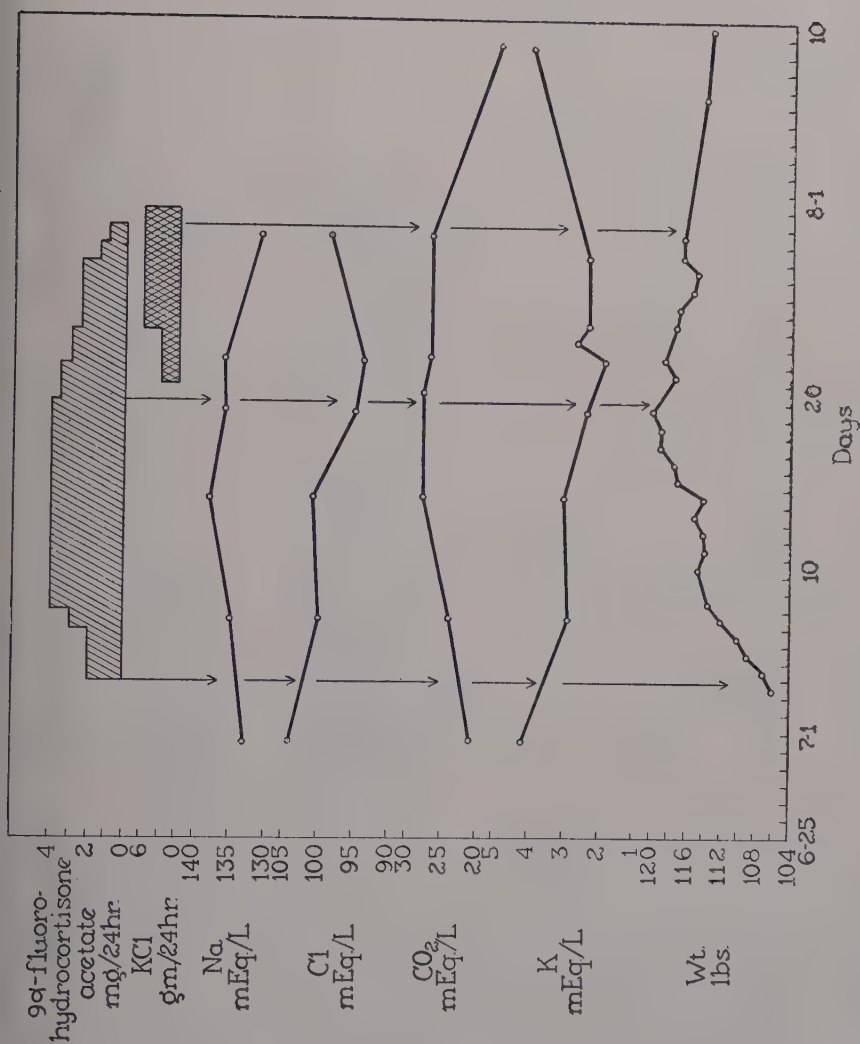


FIGURE 7 (case 3). Effect of 9α-fluorohydrocortisone acetate on serum sodium, chloride, carbon dioxide content and potassium, and on body weight.

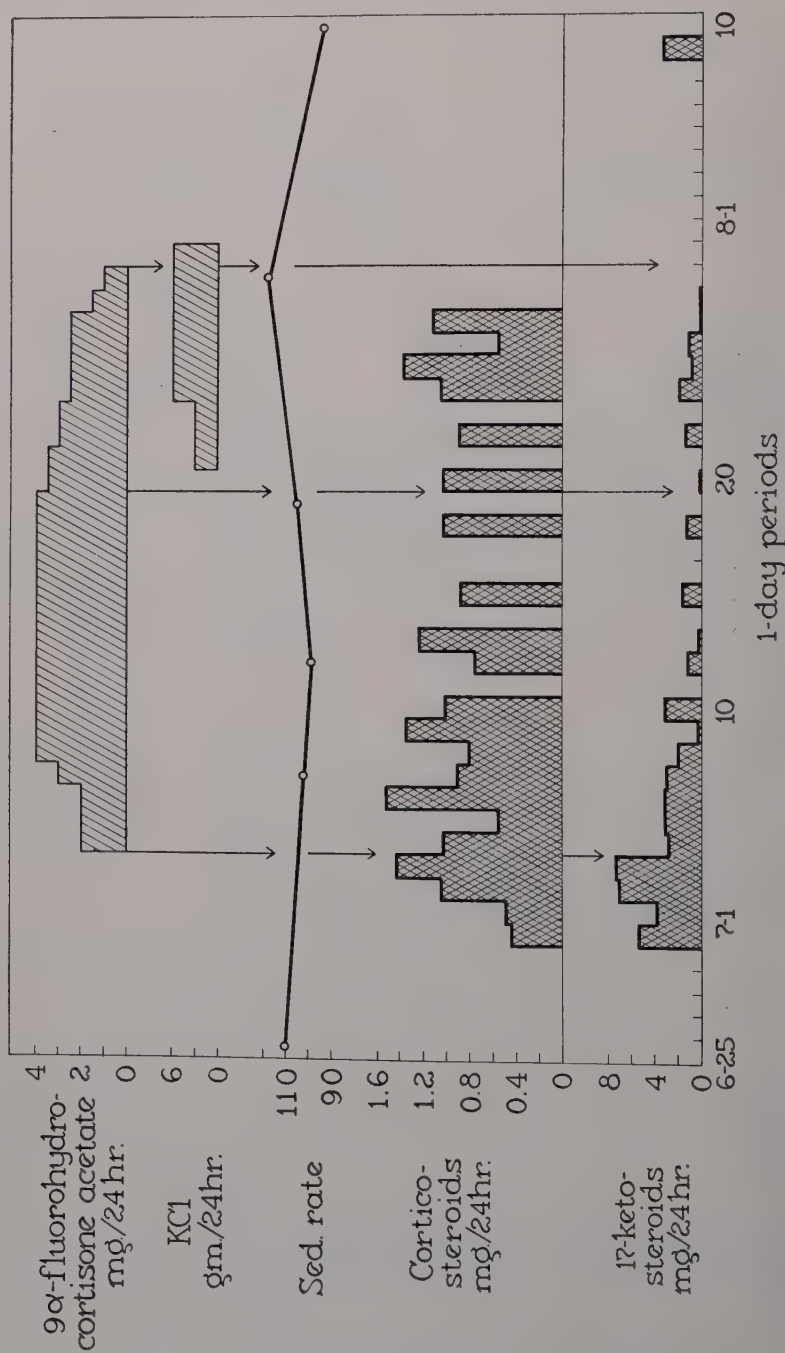


FIGURE 8 (case 3). Effect of 9α-fluorohydrocortisone acetate on sedimentation rate and on urinary excretion of corticosteroids and 17-ketosteroids.

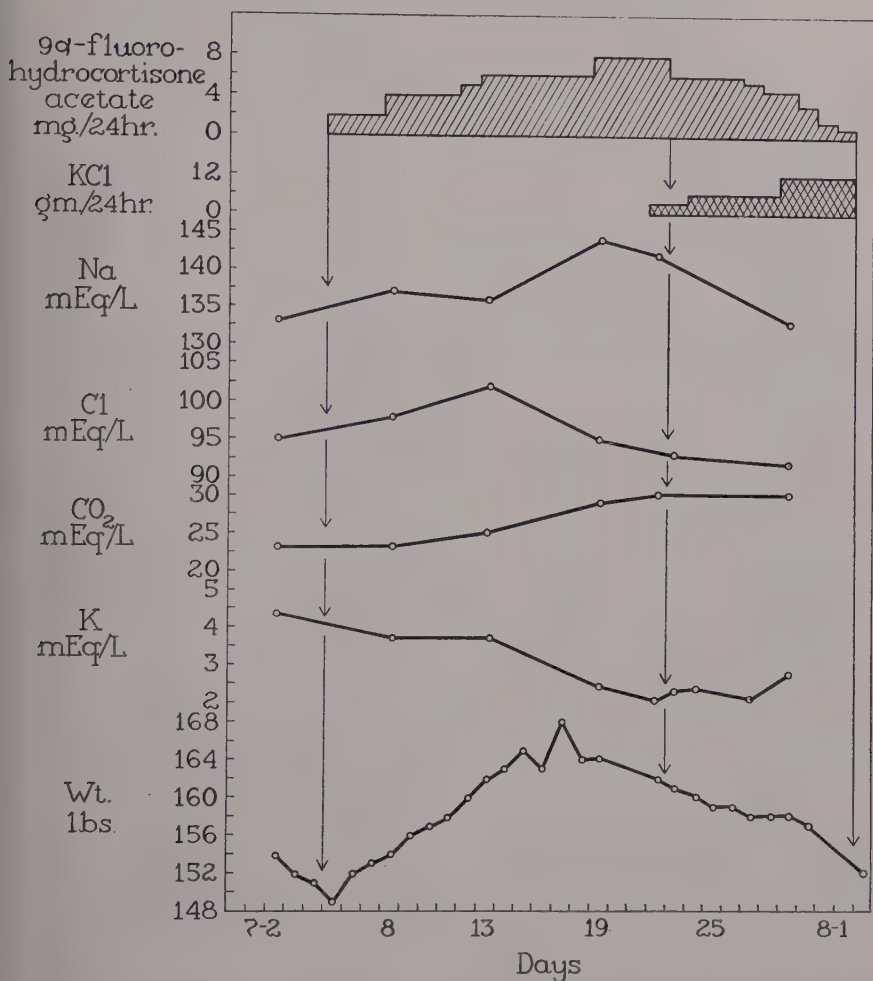


FIGURE 9 (case 4). Effect of 9α-fluorohydrocortisone acetate on serum sodium, chloride, carbon dioxide content and potassium, and on body weight.

days: 2 mg. daily for 13 days, 4 mg. daily for 12 days, and 6 mg. daily for 59 days. Doses of 2 mg. daily produced no detectable antirheumatic effect but did produce edema. Doses of 4 mg. daily produced only slight antirheumatic effect. Doses of 6 mg. daily led to marked subjective relief from, and moderate objective lessening of, the rheumatoid manifestations. Maximal gain in weight (presumably owing chiefly to retention of fluid) was five pounds. The patient noted considerable thirst during treatment. The concentration of serum potassium, which was 4.5 mEq. per liter before treatment, decreased to a minimum of 1.8 mEq. per liter during treatment, yet the patient exhibited no signs of muscular weakness.

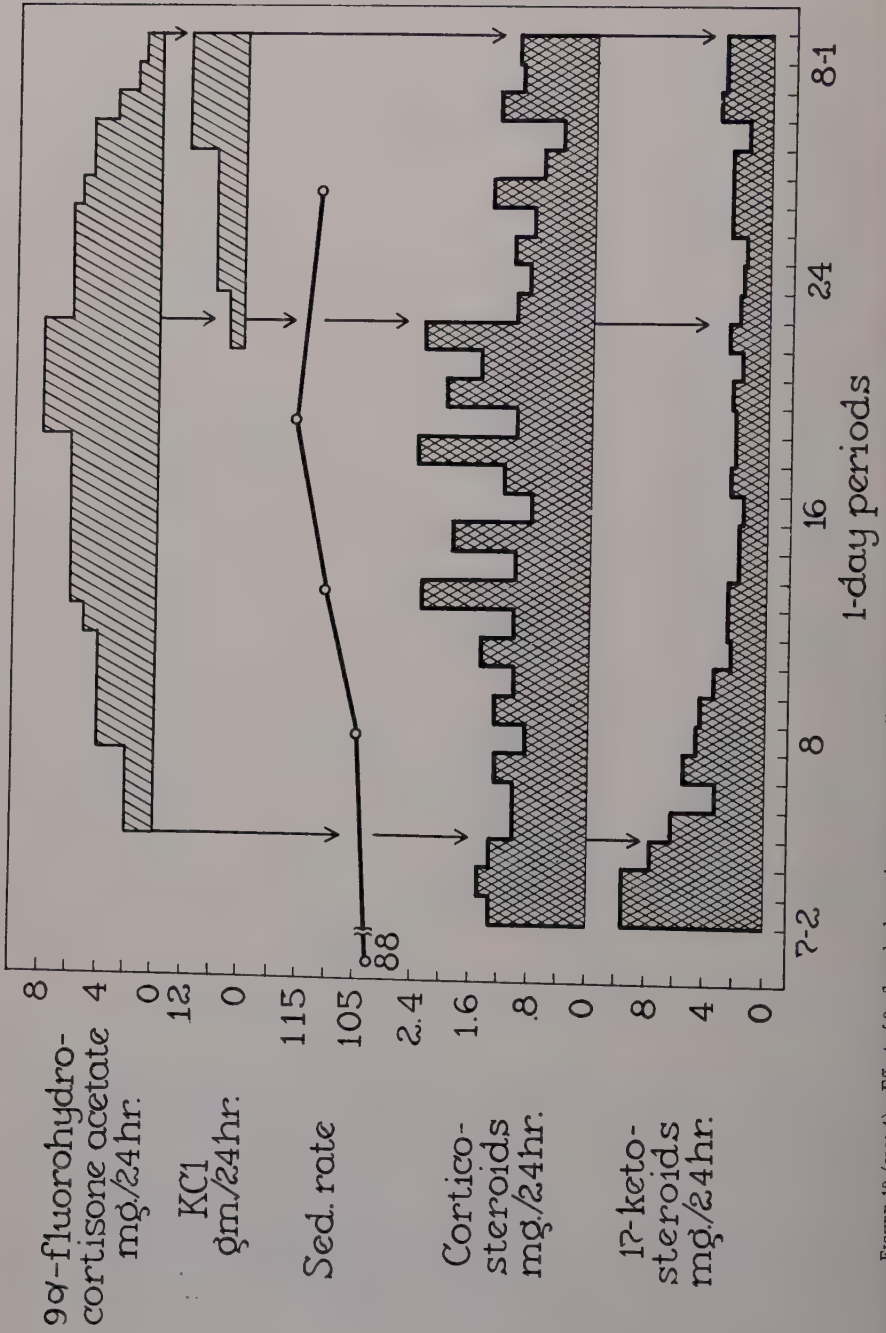


FIGURE 10 (case 4). Effect of 9α-fluorohydrocortisone acetate on sedimentation rate and on urinary excretion of corticosteroids and 17-ketosteroids.



Balance studies performed in case 2 revealed marked retention of sodium and chloride, and increased excretion of potassium, but no appreciable effect on excretion of nitrogen, calcium, or inorganic phosphate during administration of 9 $\alpha$ -fluorohydrocortisone acetate (FIGURES 5 and 6).

9 $\alpha$ -Fluorohydrocortisone acetate reduced the urinary excretion of 17-ketosteroids in the three patients tested in this regard. Hence, it would appear to inhibit pituitary-adrenocortical function to some extent.

*Antirheumatic effects.* This synthetic halogenated hydrocortisone has much greater (milligram for milligram) antirheumatic potency than that of hydrocortisone acetate, hydrocortisone (free alcohol) or cortisone acetate. Although results of treatment in these four cases varied, the small daily doses (4 to 8 mg. given in divided doses) produced about the same antirheumatic effect as one might expect from 50 to 75 mg. of cortisone acetate or from 40 to 65 mg. of hydrocortisone (free alcohol). 9 $\alpha$ -Fluorohydrocortisone acetate has been reported by others as having about 10 times more antirheumatic effect than hydrocortisone (free alcohol).

*Effect on electrolytes.* The 9 $\alpha$ -fluorine atom enhances markedly the capacity of hydrocortisone not only to suppress rheumatic symptoms but also to influence the metabolism of electrolytes and water. The antirheumatic potency of small doses, unfortunately, is usually accompanied by retention of sodium, chloride, and water, and by loss of potassium. These may cause edema and hypopotassemia with a tendency to hypochloremia and alkalosis. Although the blood pressure was not particularly affected in our cases, it has increased markedly in some cases.<sup>17</sup>

With respect to significant hypopotassemia, this developed fairly promptly in cases 2 to 5 and was reflected in the electrocardiograms, but the four patients noted no muscular weakness or other symptoms thereof. During the administration of potassium chloride in cases 3 and 4, the doses of 9 $\alpha$ -fluorohydrocortisone acetate were being reduced. Hence, the corrective effect of the potassium chloride, if any, could not be gauged. Further studies should be made to see whether symptoms attributed to hypopotassemia from cortisone and hydrocortisone also accompany the hypopotassemia from 9 $\alpha$ -fluorohydrocortisone acetate.

The enhanced effect of 9 $\alpha$ -fluorohydrocortisone acetate on electrolytes counterbalances its enhanced antirheumatic activity, lessens its usefulness as a therapeutic agent (at least for rheumatoid arthritis), and poses difficult problems for those who would give this material to patients for investigative purposes. Whether countermeasures, such as restricted intake of sodium, use of potassium and other diuretics, will permit its successful use remains to be seen.

#### *1-Dehydrocortisone (Metacortandracin)*

1-Dehydrocortisone (metacortandracin or meticorten) is a new synthetic steroid whose structure was reported recently by Herzog and associates.<sup>20</sup> Bunim and associates<sup>21, 22</sup> found it to be antirheumatic and suggested that it produces less undesired effects, particularly in respect to retention of sodium, chloride, and water, than cortisone or hydrocortisone when equivalent antirheumatic doses are compared.

*Results from use in rheumatoid arthritis.* Our results in about a dozen cases of rheumatoid arthritis confirm the potent antirheumatic effect of 1-dehydrocortisone (metacortandracin).<sup>\*</sup> We have noted moderate or marked suppression of signs and symptoms of active rheumatoid arthritis in patients treated with daily doses ranging from 7.5 to 30 mg. Oral administration was employed. Approximately equal doses were given four times daily. Our preliminary impression is that 1-dehydrocortisone is, in antirheumatic activity, approximately three or four times as potent, milligram for milligram, as cortisone, although the comparative potency varies somewhat from patient to patient.

Our experience with this compound is not yet sufficient to warrant conclusions regarding the comparative frequency and severity of undesired effects. The initial studies of Bunim and associates and our studies indicate that the action of 1-dehydrocortisone is not solely antirheumatic or anti-inflammatory, but also includes certain other cortisonelike metabolic effects. For example, administration of 30 mg. of 1-dehydrocortisone daily for 24 days to a 64-year-old man with severe rheumatoid arthritis increased the loss of nitrogen in amounts up to 2.5 gm. per day. Details of this balance study and other studies with this steroid will be reported subsequently by us and our colleagues. As with the older cortisones, careful regulation of dosage of this newest one will be necessary to avoid hypercortisonism during treatment.

#### Comment

*Antirheumatic steroids: relation of structure to activities.* About five years ago, our colleagues, Polley and Mason, reviewing our results from the use of a number of steroids, suggested that, to be antirheumatic, a steroid needed at least a ketone group at C-3 and C-20, either a ketone or hydroxyl group at C-11, a double bond between C-4 and C-5, and a hydroxyl group at C-17 and C-21.<sup>23</sup> To date, no steroid lacking these features has proved to be antirheumatic, but all steroids having them do not possess antirheumatic activity. Alterations elsewhere in the molecule may cause loss of antirheumatic effect. For example, 6-bromocortisone did not prove to be antirheumatic.<sup>24</sup>

Aldosterone (electrocortin) and corticosterone (compound B of Kendall, substance H of Reichstein) are closely related in structure; both lack a hydroxyl group at C-17. Corticosterone exhibited no antirheumatic activity when administered by us to a rheumatoid patient in doses of 500 mg. daily for 18 days.<sup>25</sup> Yet the addition of a hydroxyl group to the corticosterone molecule at C-17 results in a potent antirheumatic steroid, hydrocortisone. Perhaps, the apparent lack of antirheumatic effect of aldosterone in this study is owing to the lack of a hydroxyl group at C-17, if not to the small doses employed. 17-Hydroxyaldosterone might well be a potent antirheumatic steroid. Whether or not the effects of this compound on electrolytes would interfere with its practicality for use for arthritic patients is conjectural.

9 $\alpha$ -Fluorohydrocortisone, of course, has the structure of hydrocortisone plus a fluorine atom substituted in the  $\alpha$  position at C-9. This substitution markedly

<sup>\*</sup> The 1-dehydrocortisone (metacortandracin) used in this study was supplied through the courtesy of Doctor E. E. Henderson, Schering Corporation, Bloomfield, N. J.

enhances not only antirheumatic activity but also metabolic effects, especially in respect to retention of sodium, chloride, and water and to excretion of potassium by the kidneys. These effects lead to edema and hypopotassemia, with a tendency for the development of hypochloremia and alkalosis.

1-Dehydrocortisone (metacortandracin) differs from cortisone only in the occurrence of a double bond between C-1 and C-2. Thus, it lacks none of the features heretofore considered essential for an antirheumatic effect. Yet by virtue of an extra double bond in the ring, it achieves an enhanced antirheumatic activity. But to accomplish this, the extra double bond must be placed selectively, not indiscriminately, for the compound 6-dehydrocortisone, which differs from cortisone only in the occurrence of an extra double bond in the ring between C-6 and C-7 instead of between C-1 and C-2) was not found to have antirheumatic properties in doses employed (a total of 1 gm. in six days).<sup>23</sup>

Many interesting steroids can be conjured up mentally. What, for example, might be the physiologic activities of 17-hydroxyaldosterone, of 9 $\alpha$ -fluoro-1-dehydrocortisone, or of 9 $\alpha$ -fluoro-1-dehydro-17-hydroxyaldosterone? The list of these hypothetical steroids seems to be limited only by the imagination and the size of the paper on which to write their names and draw their configurations. It is considerably easier to name (or to nickname) these compounds than to prepare them. Therefore, the substance and the intriguing effects of these particular steroids must exist only in the mind, until chemists can synthesize them for practical testing. Perhaps, the chemists can retaliate upon clinicians who indulge in such "armchair" synthetic chemistry by introducing U<sup>235</sup> into the molecule of one of these hypothetical steroids and thereby exploding idle speculations of this sort.

*Possible dissociation of antirheumatic from other physiologic effects. Dissociation of one metabolic effect from another.* That a particular steroid may be stronger than another in producing one metabolic effect, yet weaker in producing a second metabolic effect, is not a new concept or an unproved theory but, rather, a demonstrated fact. For example, desoxycorticosterone affects the metabolism of electrolytes more than cortisone does, yet cortisone affects the metabolism of carbohydrates and proteins more than desoxycorticosterone does. Corticosterone is intermediate in these activities.

*Dissociation of antirheumatic effect from certain metabolic effects.* Many physicians have doubted seriously whether the antirheumatic effect of steroids could be dissociated from those metabolic effects which are less desirable at least for rheumatic patients. Yet definite dissociation between antirheumatic and certain metabolic effects is demonstrated by these three newly studied steroids. In brief, aldosterone has a marked action on electrolytes but no antirheumatic effect, 9 $\alpha$ -fluorohydrocortisone acetate has markedly antirheumatic manifestations and marked influence on electrolytes. 1-Dehydrocortisone has marked antirheumatic properties but less effect on electrolytes.

Whether or not the steroids 9 $\alpha$ -fluorohydrocortisone acetate and 1-dehydrocortisone prove to be of clinical usefulness, their production carries great significance for the future, because, with them, chemists have demonstrated that they can produce synthetically steroids with specialized activities, steroids which are by weight much more potent in some respects than cortisone or hydro-



cortisone. From the continued development of new steroids, there will probably eventuate superior compounds, that is, compounds with marked antirheumatic or anti-inflammatory potency, but not enough of other effects to produce unwanted manifestations.

### *Summary and Conclusions*

Aldosterone was administered intramuscularly to two rheumatoid patients for six days each in doses up to 800 and 1,000  $\mu\text{g}$ . per day, respectively. These doses produced no antirheumatic effect nor any worsening of the arthritis, but did produce some retention of sodium, chloride and water.

9 $\alpha$ -Fluorohydrocortisone acetate was administered orally to four rheumatoid patients in doses up to 4, 6, 6, and 8 mg. daily for periods of 26, 12, 84, and 28 days, respectively. These comparatively small doses lessened rheumatic symptoms significantly, but produced troublesome retention of sodium, chloride, and water and loss of potassium. The changes were sufficient to cause edema and hypopotassemia, with a tendency for the development of hypochloremia and alkalosis.

1-Dehydrocortisone (metacortandracin) was notably antirheumatic when administered orally to rheumatoid patients in daily doses ranging from 7.5 to 30 mg. In these preliminary studies, the dosage of 1-dehydrocortisone required for a given antirheumatic effect appeared to be about one fourth to one third the required dosage of cortisone. 1-Dehydrocortisone also produces effects other than antirheumatic ones. Hence, during prolonged use, its dosage should be regulated carefully to avoid hypercortisonism. In one of our cases, 30 mg. daily proved to be an excessive dose in so far as nitrogen balance was concerned. It produced a markedly negative nitrogen balance. Further studies comparing the various effects from 1-dehydrocortisone with those from cortisone or hydrocortisone are indicated.

Studies on the comparative effects of various steroids have extended our knowledge concerning the structural characteristics which give them their antirheumatic potency. It is now obvious that the chemical structure of useful antirheumatic steroids may be modified. Their desirable qualities may be maintained or even enhanced while their other qualities may be lessened. This should foster the development of superior new compounds or combinations of compounds for the control of rheumatoid arthritis and certain other diseases.

### *References*

1. SIMPSON, S. A., J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW & T. REICHSTEIN. 1953. Isolierung eines neuen kristallisierten Hormons aus Nebennieren mit besonders hoher Wirksamkeit auf den Mineralstoffwechsel. *Experientia*. **9**: 333-335.
2. MATTOX, V. R., H. L. MASON & A. ALBERT. 1953. Isolation of a sodium-retaining substance from beef adrenal extract. *Proc. Staff Meetings Mayo Clinic*. **28**: 569-576.
3. KNAUFF, R. E., E. D. NIELSON & W. J. HAINES. 1953. Studies on a mineralocorticoid from hog adrenal extract. *J. Am. Chem. Soc.* **75**: 4868-4869.
4. MASON, H. L. & V. R. MATTOX. Unpublished data.
5. LUETSCHER, J. A., JR., R. NEHER & A. WETTSTEIN. 1954. Isolation of crystalline aldosterone from the urine of a nephrotic patient. *Experientia*. **10**: 456-458.
6. REICHSTEIN, T. 1953. The main adreno-cortical hormones. *Proc. 8th Intern. Congr. Rheumatic Diseases*. Geneva, Switzerland. In press.
7. SIMPSON, S. A., J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, O. SCHINDLER & T.



- REICHSTEIN. 1954. Konstitution des Aldosterons, des neuen Mineralocorticoids. *Experientia*. **10**: 132-133.
8. WARD, L. E., H. F. POLLEY, C. H. SLOCUMB, P. S. HENCH, H. L. MASON, V. R. MATTOX & M. H. POWER. 1954. The effects of aldosterone (Electrocortin) and of 9 $\alpha$ -fluoro-hydrocortisone acetate on rheumatoid arthritis: preliminary report. *Proc. Staff Meetings Mayo Clinic*. **29**: 649-663.
9. FRIED, J. & E. F. SABO. 1954. 9 $\alpha$ -fluoro derivatives of cortisone and hydrocortisone. *J. Am. Chem. Soc.* **76**: 1455-1456.
10. BORMAN, A. & F. M. SINGER. 1954. Adrenocortical activity of 9 $\alpha$ -halo derivatives of cortisone and hydrocortisone. *Federation Proc.* **13**: 581.
11. CALLOW, R. K., J. LLOYD & D. A. LONG. 1954. A chloroderivative of cortisone with enhanced activity. *Lancet*. **2**: 20.
12. LIDDLE, G. W., M. W. PECHET & F. C. BARTTER. 1954. Enhancement of activity of hydrocortisone by substitution of a halogen atom in the 9-alpha position. *J. Clin. Endocrinol.* **14**: 813.
13. LIDDLE, G. W., M. M. PECHET & F. C. BARTTER. 1954. Enhancement of biological activities of corticosteroids by substitution of halogen atoms in 9 $\alpha$  position. *Science*. **120**: 496-497.
14. FRIED, J. & E. F. SABO. 1953. Synthesis of 17 $\alpha$ -hydroxycorticosterone and its 9 $\alpha$ -halo derivatives from 11-epi-17 $\alpha$ -hydroxycorticosterone. *J. Am. Chem. Soc.* **75**: 2273-2274.
15. LEADING ARTICLE. 1954. Diagnosis and treatment of adrenocortical disorders. *Lancet*. **2**: 26-27.
16. BOLAND, E. W. 1954. Discussion. *Proc. Am. Rheumatism Assoc. Ann. Rheumatic Diseases*. **13**: 348.
17. BOLAND, E. W. & N. E. HEADLEY. 1954. Preliminary clinical trials with 9-alpha-fluoro hydrocortisone acetate in rheumatoid arthritis. *Ann. Rheumatic Diseases*. **13**: 291-296.
18. BUNIM, J. J. 1954. Discussion. *Proc. Am. Rheumatism Assoc. Ann. Rheumatic Diseases*. **13**: 348-349.
19. BAYLES, T. B. 1954. Discussion. *Proc. Am. Rheumatism Assoc. Ann. Rheumatic Diseases*. **13**: 348.
20. HERZOG, H. L., A. NOBILE, S. TOLKSDORF, W. CHARNEY, E. B. HERSHBERG & P. L. PERLMAN. 1955. New antiarthritic steroids. *Science*. **121**: 176.
21. BUNIM, J. J., M. M. PECHET & A. J. BOLLETT. 1954. Preliminary observations on the antirheumatic potency, metabolic effects and hormonal properties of metacortandralone and metacortandracin. Presented at interim session of Am. Rheumatism Assoc. at the National Institutes of Health, Bethesda, Md. Nov. 4th.
22. BUNIM, J. J., M. M. PECHET & A. J. BOLLETT. 1955. Studies on metacortandralone and metacortandracin in rheumatoid arthritis: antirheumatic potency, metabolic effects and hormonal properties. *J. Am. Med. Assoc.* **157**: 311-318.
23. POLLEY, H. F. & H. L. MASON. 1950. Rheumatoid arthritis: effects of certain steroids other than cortisone and of some adrenal cortex extracts. *J. Am. Med. Assoc.* **143**: 1474-1481.
24. KENDALL, E. C. 1950. The development of cortisone as a therapeutic agent. *Collected Papers Mayo Clin. and Mayo Foundation*. **42**: 1-8.
25. Data unpublished by our group.



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